E & A
IMP. INST. ENT. LIBRARY
12 JUN 1939
SERIAL SEPARATE
As. 6o B

ICAR. 5. IX-2.
750

Vol. IX, Part II April, 1939

THE INDIAN JOURNAL OF AGRICULTURAL SCIENCE

Issued under the authority
of
The Imperial Council of Agricultural Research

Annual subscription
Rs. 15 or 24s.

Price per part
Rs. 3 or 5s.

Published by the Manager of Publications, Delhi
Printed by the Manager, Government of India Press, New Delhi
1939

List of Agents in India from whom Government of India Publications are available.

(a) PROVINCIAL GOVERNMENT BOOK DEPOTS.

MADRAS :—Superintendent, Government Press, Mount Road, Madras.

BOMBAY :—Superintendent, Government Printing and Stationery, Queen's Road, Bombay.

SIND :—Manager, Sind Government Book Depot and Record Office, Karachi (Sadar).

UNITED PROVINCES :—Superintendent, Printing and Stationery, U. P., Allahabad.

PUNJAB :—Superintendent, Government Printing, Punjab, Lahore.

CENTRAL PROVINCES :—Superintendent, Government Printing, Central Provinces, Nagpur.

ASSAM :—Superintendent, Assam Secretariat Press, Shillong.

BIHAR :—Superintendent, Government Printing, P. O. Gulzarbagh, Patna.

NORTH-WEST FRONTIER PROVINCE :—Manager, Government Printing and Stationery, Peshawar.

ORISSA :—Press Officer, Secretariat, Cuttack.

(b) PRIVATE BOOK-SELLERS.

Aero Stores, Karachi.*
Banthiya & Co., Ltd., Station Road, Ajmer.
Bengal Flying Club, Dum Dum Cantt.*
Bhawnani & Sons, New Delhi.
Bombay Book Depot, Charni Road, Girgaon, Bombay.
Book Company, Calcutta.
Booklover's Resort, Taikad, Trivandrum, South India.
Burma Book Club, Ltd., Rangoon.
Cambridge Book Co., Booksellers, New Dak Bungalow Road, Patna.
Chatterjee & Co., 3, Bacharam Chatterjee Lane, Calcutta.
Chukervertty, Chatterjee & Co., Ltd., 13, College Square, Calcutta.
Das Gupta & Co., 54/3, College Street, Calcutta.
Dastane Brothers, Home Service, 456, Raviwar Peth, Poona 2.
Delhi and U. P. Flying Club, Ltd., Delhi.*
Deshmukh Book Depot, Station Road, Sholapur.
English Book Depot, Ferozepore.
English Bookstall, Karachi.
English Book Store, Abbottabad, N.-W. F. P.
Faqir Chand Marwah, Peshawar Cantonment.
Higginbothams, Madras.
Hindu Library, 137-F, Balaram De Street, Calcutta.
H. L. College of Commerce, Co-operative Stores, Ltd., Ahmedabad.
Hyderabad Book Depot, Chaderghat, Hyderabad (Deccan).
Ideal Book Depot, Rajpur Road, Dehra Dun.
Imperial Publishing Coy., Lahore.
Indian Army Book Depot, Dayalbagh, Agra.
Indian Army Book Depot, Daryaganj, Delhi.
Indian School Supply Depot, Central Avenue South, P. O. Dharamtala, Calcutta.
International Book Service, Poona 4.
Jaina & Bros., Mori Gate, Delhi and Connaught Place, New Delhi, Messrs. J. M.
Kamala Book Depot, 15, College Square, Calcutta.
Keale & Co., 65, Britto Road, Karachi Sadar.
Kitabistan, 17-A, City Road, Allahabad.
Krishnaswami & Co., Teppakulam, P. O., Trichinopoly Fort, Messrs. S.
Lahiri & Co., Ltd., Calcutta, Messrs. S. K.
London Book Co. (India), Arbab Road, Peshawar, Murree, Nowshera, Rawalpindi.
Malhotra & Co., Post Box No. 94, Lahore, Messrs. U. P.
Mathur & Co., Messrs. B. S., Chatur-Vilas, Paota, Civil Lines, Jodhpur (Rajputana).
Minerva Book Shop, Anarkali Street, Lahore.
Modern Book Depot, Bazar Road, Sialkot Cantonment.
Mohanlal Dossabhai Shah, Rajkot.
Mohendra Bros., Lashkar, Gwalior State, Messrs.
New Book Co., "Kitab Mahal", 192, Hornby Road, Bombay.
Newman & Co., Ltd., Calcutta, Messrs. W.
Oxford Book and Stationery Company, Delhi, Lahore, Simla, Meerut and Calcutta.
Parikh & Co., Baroda, Messrs. B.
Pioneer Book Supply Co., 20, Shib Narayan Das Lane, Calcutta and 219, Cloth Market, Delhi.
Popular Book Depot, Grant Road, Bombay.
Punjab Religious Book Society, Lahore.
Raghunath Prasad & Sons, Patna City.
Rama Krishna & Sons, Booksellers, Anarkali, Lahore.
Ram Krishna Bros., Opposite Bisrambag, Poona City.
Ramesh Book Depot and Stationery Mart, Kashmere Gate, Delhi.
Ray & Sons, 43, K. & L. Edwardes Road, Rawalpindi, Murree and Peshawar, Messrs. J.
Roy Chowdhury & Co., 72, Harrison Road, Calcutta, Messrs. N. M.
Saraswati Book Depot, 15, Lady Hardinge Road, New Delhi.
Sarcar & Sons, 15, College Square, Calcutta, Messrs. M. C.
Sharada Mandir, Ltd., Nai Sarak, Delhi.
Standard Bookstall, Karachi.
Standard Bookstall, Quetta.
Standard Book Depot, Lahore, Delhi and Simla.
Standard Law Book Society, 79/1, Harrison Road, Calcutta.
Tara & Sons, Razmak (India), Messrs. B. S.
Taraporevala Sons & Co., Bombay, Messrs. D. B.
Thacker & Co., Ltd., Bombay.
Thacker, Spink & Co., (1933), Ltd., Calcutta.
Tripathi & Co., Booksellers, Princes Street, Kalbadevi Road, Bombay, Messrs. N. M.
Uberoy, J. C., Journalist, Printer and Publisher, Jaycee House, Alexandra Road, Ambala.
University Book Agency, Kacheri Road, Lahore.
Upper India Publishing House, Ltd., Literature Palace, Ammuddaula Park, Lucknow.
Varadachary & Co., Madras, Messrs. P.
Venkatasubban, A., Law Bookseller, Vellore.
Wheeler & Co., Allahabad, Calcutta and Bombay, Messrs. A. H.
Young Man & Co. (Regd.), Egerton Road, Delhi.

*Agent for publications on Aviation only.

CONTENTS

VOL. IX, PART II

(April, 1939)

The Editorial Committee of the Imperial Council of Agricultural Research, India, takes no responsibility for the opinions expressed in this Journal

---

		PAGE
Original articles—		
THE GENUS *FUSARIUM*, I. KNOWN OCCURRENCE IN INDIA	*G. Watts Padwick* .	171
STUDIES IN INDIAN CHILLIES: (5) INHERITANCE OF ANTHER COLOUR AND ITS RELATION TO COLOUR IN PETAL AND NODE IN *CAPSICUM ANNUUM* L.	*R. B. Deshpande, M.Ag. (Bom.), Assoc. I.A.R.I.*	185
STUDIES ON QUALITY IN RICE—		
III. *IN VITRO* DIGESTIBILITIES OF DIFFERENT VARIETIES OF RICE	*A. Sreenivasan, M.A., D.Sc., A.I.C., and K. Venkata Giri, M.Sc., Ph.D., A.I.I.Sc.*	193
IV. STORAGE CHANGES IN RICE AFTER HARVEST	*A. Sreenivasan, M.A., D.Sc., A.I.C.*	208
ENTOMOLOGICAL INVESTIGATIONS ON THE LEAF-CURL DISEASE OF TOBACCO IN NORTHERN INDIA, III. THE TRANSMISSION OF LEAF-CURL BY WHITE-FLY, *BEMISIA GOSSYPIPERDA*, TO TOBACCO, SANN-HEMP AND A NEW ALTERNATE HOST OF THE LEAF-CURL VIRUS (WITH PLATES VII-X AND ONE TEXT-FIGURE)	*Hem Singh Pruthi, M.Sc., Ph.D. (Cantab.), F.N.I. and C. K. Samuel, B. Sc.*	223
THE PHYTOPHAGOUS CHALCIDOID, *EURYTOMA SAMSONOVI* VAS., A SERIOUS PEST OF APRICOT AND SOME OTHER FRUITS IN INDIA (WITH PLATE XI)	*Hem Singh Pruthi, M.Sc., Ph.D. (Cantab.), F.N.I. and H.N. Batra, B.Sc., Assoc. I.A.R.I.*	277

		Page
Studies in the Periodic Partial Failures of American Cotton in the Punjab—		
I. Early Breakdown of the Photosynthetic Apparatus (with Plates XII-XIV)	*R. H. Dastur*	285
II. Formation and Accumulation of Tannins in Leaves (with Plates XV-XVII)	*R. H. Dastur*	291
Variations in the Measurable Characters of Cotton Fibre, I. Variation with respect to the Length of the Fibre (with eight text-figures)	*R. L. N. Iyenger, M.Sc.*	305
Studies on the Root-rot Disease of Cotton in the Punjab, VI. Chemical Composition of Healthy and Diseased Cotton Plants	*R. S. Vasudeva, B.Sc., Ph.D. (Lond.), D.I.C. (Lond.) and Mohammad Rafique, M.Sc.*	331
Note		343
Reviews—		
Soilless Growth of Plants		353
Plant Injection for Diagnostic and curative Purposes		354

ORIGINAL ARTICLES

THE GENUS *FUSARIUM*

I. KNOWN OCCURRENCE IN INDIA

BY

G. WATTS PADWICK

Imperial Mycologist, Imperial Agricultural Research Institute, New Delhi

(Received for publication on 12th September 1938)

CONTENTS

	Page
I. Introduction	171
II. Species recorded in India	172
III. Difficulties in the way of identification	176
IV. Summary	182
References	182

I. Introduction

Amongst the fungi there is probably no single genus which causes more havoc in cultivated crops than the genus *Fusarium*. In India two or three highly important diseases due to species of *Fusarium* are known. Amongst these, most work has been done on the wilt diseases of cotton and pigeon-pea, especially as regards the search for resistant varieties. It may be anticipated that in time similar work will be done on resistance in the case of other diseases due to *Fusarium*.

There is one point which has to be kept clearly in mind before any attempts are made at testing varieties of crops for resistance to disease. It is quite futile for any worker to set out to look for resistance unless he first has a good understanding whether there exist several species of *Fusarium*, and possibly several forms of a species, capable of attacking different groups of varieties of the host. There are cases known in India where more than one distinct species of *Fusarium* is capable of causing a disease. Thus for instance, it will be seen later that dry rot of potatoes (*Solanum tuberosum*) may be due to *Fusarium oxysporum*, *F. coeruleum*, *F. javanicum* v. *radicicola*, and *F. trichothecioides* and that at least two *Fusaria* are concerned with a disease of

pigeon-pea (*Cajanus cajan=C. indicus*). Experiments at Delhi have indicated that several other *Fusarium* diseases of crop plants are similarly complicated.

It therefore becomes a primary concern of the plant pathologist first of all to learn the species of *Fusarium* with which he is working, and if possible to study even the physiologic forms, before he commences on elaborate system of testing varieties for resistance.

The best way to study such a complicated genus as *Fusarium* is to have workers making a special study of the genus at large, and not restrict them to one single species. With the object of opening the way for such a study the occurrence of the genus in India known to date is reviewed here.

II. Species recorded in India

The species recorded in India have been listed in Table I. In arranging the groups within the table the authority used is Wollenweber and Reinking [1935]. The groups are listed in column 1 in the same order as Wollenweber and Reinking have used in their key, and in column 2 the fungi are listed according to the names now used by those workers. In column 3 are the names given by the authors, to whom reference is made in column 7. Columns 4, 5 and 6 give the habitat, locality, and type of disease, if any.

At the present time only six of Wollenweber and Reinking's sixteen groups are represented, though five of the six sub-groups of Elegans and Discolor are found. Only fifteen of the known sixty-five species have so far been recorded, or if varieties and forms are included, two dozen out of the known twelve dozen. Not all these fungi have definitely been related to diseases, the diseases established by experiment as being due to *Fusaria* in India being as follows :—

Top-rot of sugarcane
Foot-rot of rice
Fruit-rot of oranges
Dry-rot of potato tubers
Foot-rot of pigeon-pea
Wilt of flax
Wilt of plantain
Wilt of pigeon-pea
Wilt of cotton
Wilt of *til*
Wilt of sann-hemp
Wilt of potatoes

TABLE I

Species of Fusarium *recorded in India*

Section	Name accepted by Wollenweber and Reinking	Name given by authors	Host or habitat	Locality	Type of disease caused	Reference
Sporotrichiella	...	...	...	...	...	.....
Liseola	*F. moniliforme* Sheld.	*F. moniliforme* Sheld.	Sugarcane (*Saccharum*)	Various parts of India.	Top rot	McRae, W. [1930, 1934].
	Ditto	*F. moniliforme* Sheld. var. *majus* Wr. et Rg.	Rice (*Oryza sativa* L.)	Madras Presidency.	Foot rot	Sundararaman, S. [1935].
	Ditto	*F. moniliforme* Sheld.	Apple (*Pyrus malus*)	United Provious.	?	Mitter, J. H. [1935].
	Ditto	Ditto	Dead leaf sheath of Sorghum (*Andropogon sorghum*).	?	...	Mitra, A. [1935].
	Ditto	Ditto	Fruits of orange (*Citrus cryocarpa*).	Bengal	Storage rot of fruit.	Ghatak, P. N. [1938].
Spicarioides	...	...	...	...	...	.....
Elegans (Orthocera)	*F. lini* Bolley	*F. lini* Bolley	Linseed (*Linum usitatissimum*).	Pusa (Bihar)	Wilt	Mitra, M. [1925].
	Ditto	Ditto	Ditto	Do.	Do.	McRae, W. [1926].
	Ditto	Ditto	Ditto	Unstated.	...	Anonymous [1925].
	Ditto	Ditto	Ditto	Bombay Presidency.	...	Uppal, B. N., Patel, M. K. and Kamat, M. N. [1935].
(Constrictum)	*F. bulbigenum* Cke. et Mass. *v. lycopersici* (Brushi) Wr. et Rg.	*F. lycopersici* Sacc.	Tomato (*Lycopersicum esculentum*)	Do.	...	Ditto.
(Oxysporum)	*F. oxysporum* Schl.	*F. oxysporum* Schl.	Potato (*Solanum tuberosum*).	Do.	Dry rot of tubers.	Uppal, B. N. [1929].
	Ditto	Ditto	Ditto	Do.	...	Uppal, B. N., Patel, M. K. and Kamat, M. N. [1935].
	Ditto	Ditto	Ditto	Do.	Dry rot of tubers.	Nagpurkar, S. D. and Kulkarni, G. S. [1921].
	F. oxysporum Schl. *v. nicotianae* John.	*F. oxysporum* Schl. var. *nicotianae* Johnson.	Tobacco (*Nicotiana tabacum*).	Do.	...	Uppal, B. N., Patel, M. K. and Kamat, M. N. [1935].
	F. oxysporum Schl. *v. cubense* (E. F. Sm.) Wr. et Rg.	*F. cubense* E. F. Sm.	Plantain (*Musa* sp.)	Bengal	Wilt	Hector, G. P. [1927].

TABLE I—*contd.*

Section	Name accepted by Wollenweber and Reinking	Name given by authors	Host or habitat	Locality	Type of disease caused	Reference
	F. oxysporum Schl. *v. cubense* (E. F. Sm.) Wr. et Rg.	*F. cubense* E. F. Sm. *v. inodoratum* Brandes.	Plantain (*Musa* sp.) .	Madras Presidency.	Wilt . .	Sundararaman, S. [1930].
	Ditto . .	*F. oxysporum* Schl. f. 3 Wr.	Plantain (*Musa sapientum*).	Poona (Bombay Presidency).	...	Uppal, B. N., Patel, M. K. and Kamat, M. N. [1935].
	? *F. vasinfectum* Atk. *forma.*	*F. udum* Butler . .	Pigeon-pea (*Cajanus cajan* = *C. indicus*).	Various parts of India.	Wilt . .	Butler, E. J. [1910].
	Ditto . .	Ditto . .	Ditto . .	Berar . .	Do. .	Pearl, R. T. [1923].
	F. vasinfectum Atk. .	?	Cotton (*Gossypium* sp.)	Various parts of India.	Do. .	Shaw, F. J. F. [1914].
	? Ditto . .	?	Ditto . .	Central Provinces.	Do. .	Ajrekar, S. L. and Bal, D. V. [1921].
	? Ditto . .	?	Ditto . .	Do. .	Do. .	Ajrekar, S. L. [1926].
(Oxysporum) .	? *F. vasinfectum* Atk. .	*F. udum* Butler . .	Cotton (*Gossypium* sp.)	...	Wilt . .	Butler, E. J. [1926].
	Ditto . .	Ditto . .	(*Sesamum*) . .	...	Do. .	Ditto.
	F. vasinfectum Atk. .	*F. vasinfectum* Atk .	Sann-hemp (*Crotalaria juncea*).	Pusa (Bihar) .	Do. .	Mitra, M. [1934].
	Ditto .	Ditto . .	Ditto . .	Pusa (Bihar) and Bombay.	Do. .	Uppal, B. N. and Kulkarni N. T. [1937].
Martiella . .	*F. coeruleum* (Lib.) Sacc.	*F. coeruleum* (Lib.) Sacc.	Potato (*Solanum tuberosum*).	Bombay Presidency.	Dry rot . .	Ajrekar, S. L. and Kamat, M. N. [1923].
	Ditto .	Ditto . .	Ditto . .	Do. .	Do. .	Uppal, B. N. [1929].
	Ditto . .	Ditto . .	Ditto . .	Bombay Presidency (common).	Do. .	Uppal, B. N., Patel, M. K. and Kamat, M. N. [1935].
	F. solani (Mart.) App. et Wr.	*F. viride* (*F. solani v. medium* Wr.)	Ditto . .	United Provinces.	?	Mitter, J. H. [1935].
	Ditto . .	*F. viride* (Lechm.) Wr. renamed as *F. solani* var *medium* Wr.	Pigeon-pea (*Cajanus cajan* = *C. indicus*).	?	...	Mitra, A. [1935].
	F. javanicum Koord *v. radicicola* Wr.	*F. radicicola* Wr. .	Potato (*Solanum tuberosum*).	Bombay Presidency.	Wilt . .	Ajrekar, S. L. and Kamat, M. N. [1923].
	Ditto . .	Ditto . .	Ditto . .	Do. .	Khoka (dry) rot	Uppal, B. N. [1929].
	Ditto . .	Ditto . .	Ditto . .	Bombay Presidency (common).	Wilt and dry rot	Uppal, B. N., Patel, M. K. and Kamat, M. N. [1935].

Eupionnotes .	...	...	...	...	...	.....
Pseudomicrocera .	...	...	...	...	...	.....
Submicrocera .	...	...	...	...	...	.....
Arachnites .	...	...	...	...	...	.....
Macroconia . .	...	...	...	...	...	.....
Lateritium . .	*F. lateritium* Nees	*F. limonis* Briosi	Lemon. (*Citrus medica* var *limonis*).	Allahabad (U. P.).	?	Mitter, J.H. and Tandon, R. N. [1930].
	F. lateritium Nees var. *uncinatum* Wr.	*F. uncinatum* Wr. .	Pigeon-pea (*Cajanus cajan*=*C. indicus*).	Pusa (Bihar) .	?	Wollenweber, H. W. [1917].
	Ditto . .	*F. lateritium* Nees. var *uncinatum* Wr.	(This is the same isolate as above).	Do. .	Foot rot . .	Wollenweber, H. W. [1938].
Arthrosporiella .	*F. camptoceras* Wr. et Rg.	*F. camptoceras* Wr. et Rg.	Bajra (*Pennisetum typhoideum*. Isolated from the ear.	?	...	Mitra, A. [1935].
	F. semitectum Berk. et Rav.	*F. semitectum* Berk. et Rav.	Fruit of *Citrus medica*	?	...	Ditto.
	F. diversisporium Sherb.	*F. diversisporium* Sherb.	Petiole of a wilted leaf of *Carica papaya*.	?	...	Ditto.
	F. semitectum Berk. et Rav. v. *majus* Wr.	*F. incarnatum* (Rob.) Sacc. renamed as *F. semitectum* Berk. et Rav. v. *majus* Wr.	Shoot of *Aegle marmelos*.	?	...	Ditto.
Gibbosum	...	...	...	...	...	...
Discolor (Trichothecioides).	*F. trichothecioides* Wr..	*F. trichothecioides* Wr..	Potato (*Solanum tuberosum*).	Bombay Presidency.	Dry rot . .	Nagpurkar, S. D. and Kulkarni, G. S. [1921].
	Ditto . .	Ditto . .	Ditto . .	Do. .	Do. .	Uppal, B. N. [1929].
	Ditto . .	Ditto . .	Ditto . .	Bombay Presidency (common).	Do. .	Uppal, B. N., Patel, M. K. and Kamat, M. N. [1935].
(Neesiola) . .	...	...	...	...	...	.....
(Saubinettii) .	*F. sambucinum* Fuck .	*F. pannosum* Massee .	Living trunks of *Cornus macrophylla*.	Punjab . .	?	Butler, E. J. and Bisby, G. R. [1931].
	Ditto . .	*F. roseum* Lk. . .	Roots and stems of *Zinnia elegans*.	Bombay Presidency.	?	Uppal, B. N. Patel, M. K. and Kamat, M. N. [1935].
	F. culmorum (W. G. Sm.).	*F. culmorum* (W. G. Sm.) Sacc. v. *leteius* Sherb.	Wheat (*Triticum vulgare*).	Do. .	...	Ditto.
Ventricosum .	...	...	...	...	...	.....

In addition, one or two other diseases, such as wilt of tobacco, head-blight of wheat and fruit spotting of citrus, may reasonably be expected to have been caused by the species of *Fusarium* isolated.

Thus it is seen that seven out of the twelve definitely established *Fusarium* diseases in India are wilt diseases. Six of these seven wilts are caused by representatives of the group Elegans.

If we consider for a moment the names given by the authors and compare them with names finally adopted by Wollenweber and Reinking [1935] we are struck by the number of changes that have been made. In spite of the fact that most of the work has been done fairly recently, out of the twenty-four species, varieties and forms mentioned above, thirteen names have been changed by Wollenweber and Reinking. This means that Wollenweber and Reinking have considered these names, which have been given first by workers elsewhere, to be merely synonyms of other species of *Fusarium*.

In addition to the above there have been reports of unidentified species of *Fusarium* in India. Narasimhan [1929] described a species of *Fusarium* which causes wilt of gram (*Cicer arietinum*) but unfortunately his description is not sufficiently detailed for identification purposes. Uppal, Patel and Kamat [1935] report species of *Fusarium* on *Arachis hypogaea*, *Cicer arietinum* and *Lathyrus sativus* in Bombay Presidency. Mitter and Tandon [1930] report unidentified species of *Fusarium* at Allahabad on *Capparis sepiara*, *Cassia obtusifolia*, *Artocarpus integrifolia*, *Sorghum vulgare*, *Cucurbita moschata*, and *Pennisetum typhoideum*.

Mitra [1931] has reported isolations of *Fusarium* from cinchona. McRae [1924] obtained twelve isolates of *Fusarium* from wilted plants of gram (*Cicer arietinum*) but repeated attempts to infect gram with these isolates met with failure [Mitra, 1925].

III. Difficulties in the way of identification

It has already been pointed out that it is fundamentally essential to get correct identifications. This is needed for the guidance of the plant pathologist himself and for those who read his published work. If the importance of this is not realised from the changes in names shown in Table I, it will at any rate be obvious from an example which will be discussed here and which gives some indication of the types of difficulties met with and some clues as to how these difficulties may be overcome.

In the year 1910 after a thorough investigation of the wilt disease of pigeon-pea (*Cajanus cajan* = *C. indicus*) Butler [1910] described a fungus which he called *Fusarium udum* and which caused wilt. The name was given, as he said, without prejudice to the question whether or not it had been previously described under a different name. Later he [Butler, 1926] reached the conclusion that it could not be distinguished from *Fusarium vasinfectum*, which attacks also cotton and sesamum. In fact he was of the opinion that it

could perhaps not be distinguished even from *F. oxysporum v. cubense* (=*F. cubense*), a previously described species of the Section Elegans. Meanwhile a large amount of work was being done by McRae and Shaw, which was published as a monograph in 1933 [McRae and Shaw, 1933] on resistance of pigeon-pea to *F. vasinfectum*. Mitra [1934] worked on the *Fusarium vasinfectum* attacking sann-hemp and concluded that it could attack also pigeon-pea but not cotton. Uppal and Kulkarni [1937] however failed to repeat the findings of Mitra in that they found that *F. vasinfectum* from sann-hemp could not infect pigeon-pea or *vice versa* though they could infect their own hosts heavily. These authors raised an objection to the method used by Mitra of inoculating with wilted material of sann-hemp or pigeon-pea of the previous year's growth.

Wollenweber [1938] published results of experiments in which he isolated from pigeon-pea material sent by Butler from Pusa in 1905 yet another parasitic *Fusarium*, which causes not a true wilt but a foot-rot and which he called *F. lateritium v. uncinatum*. He isolated the fungus in 1911 not from vascular tissue but from very small sporodochia typically present on the infected roots and stem base of the plants. He again listed *F. udum* Butl. under its old name and not under the name *F. vasinfectum*.

When all these published results are considered two things now become clear :—

1. It is possible that several species of *Fusarium* may be found associated with a wilted crop like pigeon-pea.

2. The differences between isolates several of which may cause wilt of a crop and all of which may be regarded as belonging to one species may show a considerable range of variation in both morphological characters and ability to cause infection, and it is highly important to study this question.

With regard to the first point it is clear that the mere isolation of a wilt-causing organism, and its proof as a cause of wilt by laboratory experiments, must not lead to a hasty conclusion that it is the organism commonly attributed to the disease or the only one which is concerned. That can only be determined by a careful study of the fungus.

The second point raises the whole question of the value of different characters used in identification of species of *Fusarium* and it will be pertinent here to consider the general trend of thought on the species concept in relation to mycological taxonomy and *Fusarium* in particular. The identification of species of *Fusarium* today is based on a mass of data acquired by a number of workers during the past forty years. This work has been brought together in a book by Wollenweber and Reinking [1935]. These workers use in their classification morphological and physiological characters more or less indiscriminately, though tending to stress the morphological, after growing on 'standard' media prepared from plant products. Such characters as size and shape of spores are given somewhat more weight than colour of the stroma and discoloration of medium and similar characters. Some workers have

revolted against this system. Thus Hansford [1925], for instance. speaking of the Section Elegans concluded that " the present classification of the section is useless and that our present conception of a species of *Fusarium* must be revised and broadened." He was of the opinion that the size of macroconidia, the presence of sporodochia, the colour of mycelium, substratum and sporodochia, and the presence and colour of sclerotia, were highly variable and of little value for classificatory purposes. He preferred to regard all the fungi which he studied in the Section Elegans as strains of a single species. His views were supported by Butler [1929], who, enlarging on the case of *Fusarium vasinfectum*, wrote as follows :—

" In Egypt, Fahmy (according to letters received from him) has under study three strains of cotton-wilt producing fungi of the *F. vasinfectum* type : one from America which mostly attacks American varieties ; one from India restricted to Indian cottons ; and one from Egypt chiefly on the Egyptian. Morphological differences between the three strains are very slight, but there are cultural differences. If specialization of parasitism is the guide, there would seem to be as good reason for giving specific rank to the Indian strain, which refuses to attack the Egpytian cottons, as to most other species in the Elegans Section, but what is to be done with the Egyptian strain which some times attacks the Indian varieties ? " Again he says of the value of host resistance as compared with morphologic characters for species delimitation, " Hosts that are resistant under one set of conditions may become susceptible under another. Even a variation of temperature may sometimes break down resistance to infection as shown at Madison and elsewhere, and it must not be forgotten that for a parasite the host plant constitutes a large part of its environment. " In concluding he says, " If we admit that there is no such thing in nature as a pure, invariable, homogeneous species, that habit can modify the forms of a species so long as it endures, that morphological characters are less readily modified by the environment than physiological, and that convenience is often the best guide where to draw the line between different species, we may get some degree of guidance as to which of the tendencies defined above may best be followed. We will be led, I think, on the whole to prefer the morphological basis of classification and only in special cases to stress biological differences ".

The opinions of Butler were expressed at the International Congress of Plant Sciences held at Ithaca in 1926. The remarks were provoked partly by those workers, including Gaümann studying *Peronospora*, who were tending to create large numbers of new species based on host relations. His views were those of most speakers on the subject at the conference. For instance, Shear [1929, 1] based his main criticism of parasitism as a means of species delimitation on the grounds that it is rather readily acquired by fungi and that parasites and saprophytes frequently occur in the same genus. " For practical purposes therefore it appears necessary to use the term species for groups of individuals which can be separated by recognizable morphological characters, admitting that in many cases these groups are somewhat arbitrarily established and that there are intergrading forms with other species ". This, it is presumed, meets with the requirement which he expressed in another paper

[Shear ; 1929, 2] that " The essential purpose and function of nomenclature, as I understand it, is to provide a reasonably stable, convenient, uniform and accurate means of designating groups of living organisms ". Cunningham [1929] complains that the complicated nature of the technic involved in the delimitation of species by cultural characters would lead (and has led) to this work being limited to a few specialists, a defect that would become more serious as time passes. The case of the genus *Fusarium* is picked out by him as the horrible example—" For, thanks to those few pathologists who have worked with this genus, it is in such a chaotic condition that the writer believes few mycologists or pathologists would undertake the delimitation of any species placed therein ". He believes that the solution lies in more precise monographing of species of genera occurring in any one region, describing and arranging the species according to morphological characters.

Stakman [1923] considers that physiologic characters are of equal value with morphological ones in delimiting species. In his experience with *Puccinia graminis* he found the physiological character of host specialization quite as fixed and stable as any morphological characteristic. He pointed out that most of the work done on bridging hosts was invalidated by the fact that the workers used mixed physiologic forms, so that the so-called bridging host phenomenon can no longer be held up as proof of the instability of host reactions. " If we are seeking the ultimate truth regarding fungi, surely we ought to accept it in plant behaviour as well as in plant structure ". He emphasised the importance, however, of using a pure line of the host.

Important contributions have recently come from Michigan, and their revolutionary ideas need careful consideration. Coons and Strong [1931] worked out a system of identifying a large number of *Fusaria* by their reaction to certain aniline dyes and copper compounds in artificial culture media. The important characteristics used after growing the cultures on the standard media were colours of mycelium, breadth of mycelial mat, changes in colours of medium, type of edge of the colony, and growth form. There were two colours of mycelium, red and white (a third has since been added, brown) ; various sizes of the colony ; four categories under change in medium colour ; three types of colony edge ; and nine different ' growth forms ' of mycelium. Based on these differences a key for the large number of *Fusaria* studied was prepared. According to these workers there was a " striking parallelism in the independent classification of the cultures by a *Fusarium* specialist (Sherbakoff) and the dye reactions ". Hansford [1926], Brown [1928], Mitter [1929] and others had criticised the present basis of classification of *Fusaria* on grounds of high variability within the ' species ', and Coons and Strong said " If the assignment, as done by Sherbakoff, of members of the Elegans Section into species were unreliable, to take only this section as an example, and resulted in divergent forms such as *F. cubense* and *F. oxysporum*, and others of the Elegans Section being ' not satisfactorily divided up ' as Hansford states is the true situation when present methods of classification are attempted, it would seem that contradictions in the dye response would have been inevitable ".

With this publication, then, there did not seem to be much doubt about the point of view of the Michigan workers. Whatever they might do in the way of providing a simple key by which workers in the genus might readily get a clue as to the identity of the organisms they were working with, they considered that on the whole the views of Sherbakoff on classification, and therefore inconsequence those of Wollenweber and others to whose school Sherbakoff belonged, were acceptable and that the fears of Hansford, Brown, Mitter, Butler and others were unfounded. What was wanted was not a new classification, but a short-cut to identification. Names of species would presumably remain unaltered.

The second paper from Michigan, that by Nelson, Coons and Cochran [1937] dashes to the ground any hopes, to which the first paper might have given birth, that the present classification was to be stabilised. No longer is the old system upheld. In fact, " it is extremely doubtful if this system is workable when applied to the separation of pathogenic and saprophytic *Fusaria* in the sub-section Orthocera ". Hansford's views, previously rejected, are now thrown into the front line of attack, for he is stated to have shown that *Fusarium cubense* is extremely variable morphologically and to have criticised sharply the elaborate system of classification in use. It is not mentioned whether Brown and Mitter are brought back into favour in like manner, but the admission of the unreliability of morphological characters would presuppose this. *Fusarium orthoceras* var. *apii* Wall and Rg. is no more. *Fusarium apii* and *F. apii* var. *pallidum* now rise up, distinguished by their host relationships.

We read " The authors believe that the celerypathogenes differ sufficiently from other members of the Orthocera group to constitute a new species. The most important differential character is the distinct host relationship and it is chiefly on this basis that the segregation is made ". Yet of the two varieties it is said " Because of their morphological similarities, these pathogenes are best considered as belonging to one species ". These two statements clearly mean.—

1. That these two fungi are species separate from all other species of *Fusarium* because of their host reaction.

2. That although in their host reactions the two fungi are somewhat different, they belong to the same species because they are morphologically alike !

Such is the present state of confusion in the genus *Fusarium*. It is fashionable to point out the wretched genus as one of the black sheep in a bad family. It is fashionable to demonstrate how variable is the genus *Fusarium*, and to criticise Wollenweber's classification, yet his help in identification is keenly sought. For after all he has reduced 1100 or so *Fusaria* to the very modest total of 144 including varieties or forms, he has prepared a synonymy, and the pathologist and mycologist alike are eternally grateful.

It seems that if we are to make still more improvements, we must think of the genus *Fusarium* in terms of related groups and of systematics in general. The prevalent idea of a species is well expressed by Gates [1938]. He classifies the criteria of a Linnean species into morphological differences in the phenotype and its chromosome number and their morphology, physiological and chemical differences, and differences in ecology and geographical distribution. He agrees that all Linneons need not answer all these requirements, but insists that " In the practical work of describing and dealing with species the only essential, however, is a sufficient number of morphological differences." If we are to treat *Fusarium* on a similar level with higher plants and with other fungi, our first essential for species delimitation is " a sufficient number of morphological differences ".

There can be no doubt about the high degree of variability of certain morphological characters, including those such as spore size and septation. A study of this type of variation is being made by Mme Raillo [1935]. But the view that certain physiological characters are less variable seems to be at present hardly acceptable. Samuel and Greaney [1937] recently showed that wide variation existed in the extent to which thirteen isolates of *F. culmorum* collected from wheat fields in England caused root-rot of wheat in controlled experiments, the average disease rating varying from nine to forty. The writer has further observed that many cultures of *Fusarium culmorum* and *F. avenaceum* have a wide host range. It may be that in the Section Elegans specialization is of a higher order, but even if this is proved it remains essential that throughout the genus any particular type of character should have the same general significance of rank. Host range cannot be taken for species value in the case of sections Discolor and Roseum, and it seems discordant to elevate it to specific rank in the case of the Section Elegans.

It is sometimes held that the only use for systematics is to find a pigeon hole bearing a name for the plant under consideration, that it is a mere tool. This is a confusion of ideas. The whole Linnean system, to which the plant pathologist still clings, is based, imperfect though it may be, on the idea of relationship in its broadest sense. A systematic arrangement is an entirely different thing from a key, as seemed at first to be realized by Coons and Strong. If we can rapidly form the opinion that a certain fungus is *Fusarium orthoceras* var. *apii* or *F. apii* or whatever it may be called because it attacks celery, this short-cut is very convenient. Much time is saved by such a device, and confirmation of the identity by studying other characters is grealty simplified. But this has nothing to do with systematics.

One cannot form opinions on the taxonomy of an organism without studying all closely related forms. Yet the worker in Northern Europe cannot compare the pathogenicity of *Fusarium* species of the Section Elegans on, say, bananas or cotton. Even in the crops which can be grown in various countries pathogenicity is closely bound up with variable factors such as temperature and moisture, as has been clearly shown by workers on various diseases at Wisconsin, and soil conditions as shown by Mundkur [1936] working with

cotton wilt in India. The delimitation of species on the basis of host reaction demands the use of a pure line of the host and a definition of the conditions—probably an impossibility.

One can only conclude that in the end it will be necessary to base specific rank upon characters easily recognizable under standardized conditions available in all moderately equipped mycological laboratories. This precludes the use of host relationships, which must be given a rank lower than species. In the meantime practical problems have to be solved and until something better arises, there seems nothing for it but to try to stick to Wollenweber's system. Inadequate though it may be, there is nothing better. The creation of new specific names at least should be done with the greatest caution until an acceptable alternative is constructed.

IV. Summary

(1) A primary consideration in selecting varieties of crops resistant to diseases caused by species of *Fusarium* is the determination of the organism or organisms responsible.

(2) The species of *Fusarium* recorded in India are listed. Twenty-four species or varieties are found belonging to six groups. Twelve diseases have been definitely proved to be caused by *Fusarium* and seven of these are wilt diseases. Six of these seven wilts are caused by *Fusarium* species of the Section Elegans.

(3) The importance of correct identification, and some of the reasons why this is difficult, is brought out by consideration of the case of the *Fusarium* species attacking pigeon-pea (*Cajanus cajan*), cotton (*Gossypium* spp.), *til* (*Sesamum indicum*) and sann-hemp (*Crotalaria juncea*).

(4) The value of physiologic characters such as host relationships in the species concept is discussed with special reference to *Fusarium*.

(5) It should be the ultimate aim to improve the classification of the genus *Fusarium* but in the meantime it is desirable to accept that of Wollen weber and his associates, creating as few new species as possible, and avoiding the use of any new biological characters, such as host reaction, for species delimitation.

REFERENCES

Ajrekar, S. L. (1926). *J. Ind. Bot. Soc.* **5**, 1-8

Ajrekar, S. L. and Bal, D. V. (1921). *Agric. J. Ind.* **16**, 598-617

Ajrekar, S. L. and Kamat, M. N. (1923). *Agric. J. Ind.* **18**, 515-520

Anonymous (1925). Fungi received at the Imperial Bureau of Mycology. List I. Supplement to *Rev. Appl. Myc.*

Brown, W. (1928). *Ann. Bot.* (*Lond.*) **42**, 285-304

Butler, E. J. (1910). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **2**, No. IX

———————(1926). *Agric. J. Ind.* **21**, 268-273

———————(1929). *Proc. Int. Congr. Plant Sci.* (*Ithaca*) 1926, **2**, 1590-1597

Butler, E. J. and Bisby, G. R. (1931). *Sci. Monogr. Imp. Coun. Agric. Res. Ind.* No. **1**

Coons, G. H. and Strong, M. C. (1931). *Tech. Bull. Mich. Agric. Expt. Stn.* No. **115**

Cunningham, G. H. (1929). *Proc. Int. Congr. Plant Sci.* (*Ithaca*) 1926, **2**, 1713-1717

Gates, R. R. (1938). *Amer. Nat.* **72**, 340-349

Ghatak, P. N. (1938). *J. Ind. Bot. Soc.* **17**, 141-148

Hansford, C. G. (1925). *Proc. W. Ind. Agric. Conf.* (1924), 41-51

——————————— (1926). *Kew Bull.* **7**, 257-288

Hector, G. P. (1927). *Rep. Dept. Agric. Beng.*, 1926-27 (Appendix I, 35-38)

McRae, W. (1924). *Sci. Rep. Agric. Res. Inst. Pusa*, 1923-24 : 48-49

———————— (1926). *Sci. Rep. Agric. Res. Inst. Pusa*, 1925-26 : 65

———————— (1930). *Sci. Rep. Agric. Res. Inst. Pusa*, 1928-29 : 58

———————— (1934). *Sci. Rep. Agric. Res. Inst. Pusa*, 1932-33 : 6-7

McRae, W. and Shaw, F. J. F. (1933). *Sci. Monogr. Imp. Coun. Agric. Res. Ind.* No. **7**

Mitra, A. (1935). *Ind. J. Agric. Sci.* **5**, 632-637

Mitra, M. (1925). *Sci. Rep. Agric. Res. Inst. Pusa*, 1924-25 : 52

———————— (1931). *Sci. Rep. Agric. Res. Inst. Pusa*, 1929-30 : 65

———————— (1934). *Ind. J. Agri. Sci.* **4**, 701-714

Mitter, J. H. (1929). *Ann. Bot. Lond.* **43**, 379-410

——————————— (1935). *Int. Bull. Pl. Prot.* **9**, 177M

Mitter, J. H. and Tandon, R. N. (1930). *J. Ind. Bot. Soc.* **9**, 190-198

Mundkur, B. B. (1936). *Proc. Ind. Acad. Sci.* **3**, 498-501

Nagpurkar, S. D. and Kulkarni, G. S. (1921). *Bull. Dept. Agric. Bombay* No. **102**, 57-68

Narasimhan, R. (1929). *Yearbook Dept. Agric. Madras*, 1928 : 5-11

Nelson, R., Coons, G. H. and Cochran, L. C. (1937). *Tech. Bull. Mich. Agric. Exp. Stn.* No. **155**

Pearl, R. T. (1923). *Rept. Dept. Agric. Cent. Prov. Berar* 1921-22 : 19-20

Raillo, Mme A. I. (1935). *Bull. Pl. Prot. Leningr., 1935, Ser. 2* (*Phytopath*). **7**, 5-100 (Abstract seen in *Rev. Appl. Myc.* **15** ; 684-685, 1936).

Samuel, G. and Greany, F. J. (1937). *Trans. Brit. Myc. Soc.* **21**, 114-117

Shaw, F. J. F. (1914). *Rept. Agric. Res. Inst. Pusa*, 1913-14 ; 54-55

Shear, C. L. (1929). *Proc. Int. Congr. Plant Sci.* (*Ithaca*) 1926 : 1618-1628

———————— (1929). *Proc. Int. Congr. Plant Sci.* (*Ithaca*) 1926 : 1657-1660

Stakman, E. C. (1923). *Amer. J. Bot.* **10**, 239-244

Sundararaman, S. (1930). *Admin. Rept. Govt. Mycologist, Madras*, 1929-30 : 23

——————————— (1935). *Int. Bull. Pl. Prot.* **9**, 177M

Uppal, B. N. (1929). *Rept. Dept. Agric. Bombay*, 1927-28 : 203-206

Uppal, B. N. and Kulkarni, N. T. (1937). *Ind. J. Agric. Sci.* **7**, 413-442

Uppal, B. N., Patel, M. K. and Kamat, M. N. (1935). *Bull. Dept. Agric. Bombay* No. **176**, 31

Wollenweber, H. W. (1917). *Ann. Mycol. Berl.* **15**, 1-56

——————— (1938). *Arb. Biol. Reichsanst* (Berl.) **22**, 339-347

Wollenweber, H. W. and Reinking, O. A. (1935). " Die Fusarien, ihre Beschreibung, Schadwirkung und Bekampfung ". Berlin : Paul Parey

STUDIES IN INDIAN CHILLIES

(5) INHERITANCE OF ANTHER COLOUR AND ITS RELATION TO COLOUR IN PETAL AND NODE IN *CAPSICUM ANNUUM* L.*

BY

R. B. DESHPANDE, M.Ag., (Bom.), Assoc. I.A.R.I.

Botanical Section, Imperial Agricultural Research Institute, New Delhi

(Received for publication on 30th August 1938)

CONTENTS

		Page
I.	Introduction	185
II.	Experimental results	186
III.	Theory	190
IV.	Application of theory to observed results	190
V.	Discussion	191
VI.	Summary	192
	References	192

I. Introduction

The most common colour in anthers of Indian types of chillies is either blue or purple with occasional streaks or patches of yellow. The intensity and extent of colour may, however, vary in different types.

In order to study the mode of inheritance of anther colour, the Pusa Types 3 and 29 [Shaw and Khan, 1928]—the former having bluish purple anthers and the latter bluish with yellow streaks—were crossed with a yellow-anthered mutant, T 11A.

While the F_3 of the cross T 3 × T 11A was being studied it was observed that the purple or blue anthers were associated with purplish nodes and the yellow anthers with green nodes. Though the original idea was to study only the inheritance of colour in anther, complete observations on a part of the F_2 population with regard to colour in anther, petal and node being available, it is possible to bring out interesting facts regarding the relationships between anther colour and colour in petal and node.

* Paper read at the Silver Jubilee Session of the Indian Science Congress, January, 1938

II. Experimental results

Cross T 3 × T 11A

T 3 has bluish purple anthers, purple petals and purple nodes and T 11A has yellow anthers, white petals and green nodes. In F_1, the anthers and nodes were light purple and the petals partially purple, *i.e.*, white with purple streaks. In F_2, a good deal of variation was observed in these characters, and particularly in the anther colour, suggesting that they were controlled by more than one factor. The plants with coloured anthers, whatever the intensity or extent of colour, had invariably purple nodes. The petal colour, however, segregated independently. The following frequencies were observed in F_2 :—

	Purple anthers and purple nodes (various grades)	Yellow anthers and green nodes	Total
T 3 × T 11A	252	78	330
T 11A × T 3	330	87	417
Total observed	582	165	747
Total expected on 3 : 1 basis	560·25	186·75	747

$\chi^2 = 3{\cdot}39$; P between 0·1 and 0·05. The fit is fair.

Anther and node colour frequencies in relation to petal colour

	Anthers and nodes purple		Anthers yellow, nodes green	Total
	Petal purple	Petal white	Petal white	
Total observed	133	24	41	198
Total expected on 9 : 3 : 4 basis	111·6	37·2	49·6	198·4

$\chi^2 = 10{\cdot}4$; P less than 0·01

The fit is bad. The bad fit and the absence of cultures breeding pure for ' white petals and purple anthers and nodes ' in F_3 necessitated the examination in detail of the segregations in the thirteen F_3 cultures behaving like F_2. The segregations and the values of χ^2 are given below :—

F_3 culture No.	Anthers and nodes purple		Anthers yellow, nodes green	Total	χ^2
	Petal purple	Petal white	Petal white		
1	25	4	5	34	4·14
2	27	3	16	46	5·30
1—18	32	4	6	42	6·81
1—25	29	4	7	40	4·40
1—45	27	4	8	39	2·98
1—55	22	5	12	39	1·24
1—76	31	5	9	45	3·13
1—142	30	4	8	42	3·30
1—205	26	3	10	39	4·23
2—23	26	5	13	44	2·12
2—98	28	4	8	40	3·07
2—106	28	4	7	39	3·95
2—113	26	5	5	36	3·87
Total .	357	54	114	525	Very high

' P ' being higher than 0·05 in all cases except in culture No. 1-18, the fits are good. The fit for the total of all the cultures is very poor. This is due to the phenotype ' white petals, purple anthers and nodes ' being consistently deficient.

The striking thing about these segregations is that in all the thirteen families the phenotype ' white petals and purple anthers and nodes' is deficient.

This points out that some lethal factor is operating which also may be responsible for the absence of cultures breeding pure for ' white petals and purple anthers and nodes '.

In F_3 the various cultures showed the following behaviour :—

No. of cultures in F_3 and nature of parent plant in F_2	F_3 behaviour	Formulae	Frequencies	
			Observed	Expected
11 cultures from plants with deep purple petals, anthers and nodes	Pure	**SSAABB SSAABb SSAAbb**	11	11
27 cultures from plan s with petals, anthers, and nodes like F_1 in colour	Petals, anthers and nodes purple / White petals, anthers and nodes purple. 3 : 1	**SSAaBB SSAaBb**	3	6·75
	Petals, anthers and nodes purple. / White petals, yellow anthers and green nodes. 3 : 1	**SsAABB SsAABb**	11	6·75
	Like F_2 . . .	**SsAaBB SsAaBb**	13	13·50
11 cultures from plants with white petals, and purple anthers and nodes	Pure	**SSaaBB**	0	3·66
	White petals, and purple anthers and nodes / White petals, and yellow anthers and green nodes 3 : 1	**SsaaBB SsaaBb**	11	7·32
7 cultures from plants with white petals, yellow anthers and green nodes	Pure	**ssaaBB ssaaBb ssaabb**	7	7

The results are fairly satisfactory. The absence of cultures breeding pure for white petals with purple anthers and nodes may be due to the combination, homozygous **S** and **B**, being lethal as has been pointed out earlier,

Cross T 29×T 11A

The second cross studied in this connection was between Types 29 and 11A. Both the parents have white petals, and there is thus no segregation for petal colour. T 29 has purple colour in nodes and anthers, but the anthers are neither so completely nor so deeply purple as in T 3. The yellow colour shows itself at places, particularly towards the base. T 11A has yellow anthers and green nodes. In F_1 the anthers were yellow with light purple tips. The nodes were light purple. In F_2 no plant with yellow anthers (like T 11A) and purple nodes, or *vice versa*, was observed. The following were the observed frequencies in F_2 :—

	Anthers and nodes purple like T 29	Anthers and nodes purple like F_1	Anthers yellow and nodes green like T 11A	Total
Total observed . . .	157	298	177	632
Total expected on 1 : 2 : 1 basis	158	316	158	632

$\chi^2=3\cdot30$; P between $0\cdot2$ and $0\cdot1$. The fit is good.

In F_3 the following segregations were observed :—

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulae	Frequencies	
			Observed	Expected
6 cultures from plants with anthers and nodes like Type 29	Pure . .	SSaabb .	6	6
9 cultures from plants with anthers and nodes like F_1 .	Like F_2 .	Ssaabb .	9	9
1 culture from plants with yellow anthers and green nodes (T 11A)	Pure . .	ssaabb .	1	1

The F_3 results are in agreement with the F_2 observations. The recessive nature of yellow anthers and green nodes having been established in the first cross, only one F_2 plant like Type 11A was carried to the F_3.

III. Theory

In order to explain the foregoing results it is necesary to assume that :—

S=a factor for the production of purple colour in the plant except in petals. It also converts yellow anthers to yellowish purple and green nodes to purple. When **S** is absent, no purple colour will manifest itself even when other colour factors are present.

A=a factor which produces purple colour in petals if **S** is present, *i.e.*, **S** and **A** are complementary for colour in petals.

B=a factor which intensifies purple colour in the presence of **A**.

The factorial constitution of the parents and the F_1's is as follows :—

Type 3	**AABBSS**
Type 29	**aabbSS**
Type 11A	**aabbss**
T 3 × 11A,F_1	**AaBbSs**
T 29×11A,F_1	**aabbSs**

IV. Application of theory to observed results

With regard to the colour in anthers, petals and nodes in the cross, Type 3×Type 11A, since Type 3 is homozygous for the presence of **S**, **A**, and **B**, and Type 11A lacks all these, the F_1 is heterozygous for all the three factors and has purple petals, anthers, and nodes. In F_2 the segregation of these three factors should result as follows :—

SAB	**SAb**	**SaB**	**Sab**	**sAB**	**sAb**	**saB**	**sab**
27	9	9	3	9	3	3	1
Pr. petals, anthers and nodes		White petals, pr. anthers and nodes		White petals, yellow anthers and green nodes			
36		12		16			
or 9		3		4			

This is actually observed in F_2 (page 186).

Considering the plants with purple anthers and nodes against those with yellow anthers and green nodes, a 3 : 1 ratio is expected which is realized in F_2.

The genotypes of the above phenotypes with their F_3 behaviour are given below :—

Genotypes of 27 **SAB**	Behaviour in F_3
1 **SSAABB** . .	Breed pure for deep purple petals, anthers and nodes.
2 **SSAABb** . .	
2 **SSAaBB** . .	Segregate into 3 purple petals, anthers and nodes : 1 white petals, purple anthers and nodes.
4 **SSAaBb** . .	
2 **SsAABB** . .	Segregate into 3 purple petals, anthers and nodes : 1 white petals, yellow anthers and green nodes.
4 **SsAABb** . .	
4 **SsAaBB** . .	Like F_2.
8 **SsAaBb** . .	
Genotypes of 9 **SaB**	
1 **SSaaBB**	Breed pure for white petals and purple anthers and nodes.
2 **SSaaBb** . .	
2 **SsaaBB** . .	Segregate into 3 white petals, purple anthers and nodes : 1 white petals, yellow anthers and green nodes.
4 **SsaaBb** . .	

All the other phenotypes will breed true to white petals, yellow anthers and green nodes, as they lack **S**, the basic factor for production of purple colour in plant.

Excepting the failure to realize cultures breeding pure for white petals with purple anthers and nodes, and a slightly bad fit in F_2, the observed results agree with the theory.

In the other cross, T 29×T 11A, the segregation of **S** should result as follows :—

SS	:	**Ss**	:	**ss**
1		2		1
Like T 29		Like F_1		Like T 11A

This is realized (page 189).

In F_2 the parental types should breed true and the plants like F_1 should segregate like F_2. This also is realized (Page 189).

V. Discussion

The results of both the crosses indicate that yellow colour in anther of Type 11A is recessive to the two types of purple colours present in the anthers of Type 3 and Type 29 respectively, and that the purple colour in anther and that in the node are determined by the same factor, **S**.

A good deal of variation in the itensity and extent of purple colour in the anthers and nodes of F_2 plants of the cross, Type 3 × Type 11A, indicated that the two parents differ in more than one factor. In addition to this the

appearance of plants with white petals and purple anthers and nodes in F_2 suggested further complications in their inheritance. The precise nature of the mode of inheritance of these characters became clear only during the course of the F_3 study. On referring back to the F_2 data and examining the relation of anther and node colour to petal colour, it was observed that at least three factors were concerned in determining the colour in petals, anthers, and nodes in this cross. It was then for the first time observed that a factor, termed **S**, was necessary for producing purple colour in any plant organ in addition to the other colour factors.

The study of the inheritance of the anther and node colour in the cross, T 29 × T 11A, was simple, the results showing a monohybrid segregation. The only factor in which the parents differ in this cross is **S**, which is both the basic factor for purple colour and the factor for colour in anthers and nodes.

In this connection a reference to the results of the cross T 3 × T 29, so far as they refer to the colour in anthers and nodes [Deshpande, 1933], seems necessary. The parents have been already described. The F_1 was practically like the F_1 of T 3 × T 11A, that is, purple (partially) for petals, anthers and nodes. In F_2 the T 29 form of petals, anthers and nodes was recovered as the recessive (one-fourth of the total F_2 population). This phenotype was similar to the phenotype with white petals, purple anthers and nodes realized as three-sixteenths of the F_2 population in the cross T 3 × T 11A in the present study. Moreover the F_2 plants segregating in F_3 into ' plants with purple anthers and nodes ' and ' plants with yellow anthers and green nodes ' resembled the F_1 of the cross T 29 × T 11A. Thus a relationship in these three crosses so far as the colour in petals, anthers and nodes is concerned, has been traced.

On the evidence furnished by the results of the present investigations, necessary modifications have been made in the genetic formulae previously proposed for types 3 and 29 [Deshpande, 1933]. The original results, however, are not affected by this modification.

VI. Summary

1. It is observed that the manifestation of purple colour in any organ in the plant is dependent upon the presence of a factor, **S**.

2. Yellow anthers of T 11A are recessive to the yellowish purple anthers of T 29 and to deep purple anthers of T 3 on a 1 : 3 ratio.

3. Segregation of anther and node colour in relation to petal colour was found to be on a 36 : 12 : 16 trihybrid ratio.

4. The factor **S** determines the purple colour both in anthers and nodes.

REFERENCES

Deshpande, R. B. (1933). *Ind. J. Agric. Sci.* **3**, 219-300

Shaw, F. J. F. and Khan, A. R. (1928). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **16**, No. 2

STUDIES ON QUALITY IN RICE

III. *IN VITRO* DIGESTIBILITIES OF DIFFERENT VARIETIES OF RICE

BY

A. SREENIVASAN, M.A., D.Sc., A.I.C.

AND

K. VENKATA GIRI, M.Sc., D.Sc., A.I.I.Sc.

Department of Biochemistry, Indian Institute of Science, Bangalore

(Received for publication on 13th August 1938)

It is well known that starches of different cereals are generally distinguishable from one another by the size, shape and optical properties of their granules. It has been shown that they may also be differentiated by their unequal resistance towards amylolytic enzymes and in possessing different liquefaction or gelatinisation temperatures. Among the numerous publications showing the differential effects of amylases upon starches from various sources such as wheat, barley, maize, rice, potato, etc., mention may be made of the researches of Stone [1896], O'Sullivan [1904], Wollf and Fernbach [1907], Hammerstein [1908], Reinitzer [1909], Lang [1910], Nagao [1911], Pauletig [1917], Welzmuller [1921], Giri [1935], and others. The digestibility of the starch from any particular source would also naturally depend on its method of preparation, extent of disintegration, etc., although very little systematic work would appear to have been done on this subject.

Rice is particularly rich in starch and there are a very large number of varieties in existence. Although in experiments on the digestibilities of the starches of rice varieties, the differences to be looked for would not be so marked as those found by experimenters who have compared starches from very different sources, it may yet be probable that the ease of digestion of the carbohydrate and protein constituents in rice may vary with different varieties. Indeed, in practice, there is a general preference exercised by a large section of the consumers for the finer and white varieties of rice compared to the so-called coarser types. Further, there is a belief that polished rice is more easily digested than unpolished rice ; parboiled rice is also generally considered to be more sustaining than raw rice. Scientific study in regard to the digestibility of rice varieties has, however, been wanting.

In two recent communications, Basu and co-workers [1935, 1936] have studied the digestibility by taka-diastase, ptyalin and pancreas-amylase of the starches of different varieties of Bengal rice subjected to different treatments. The present inquiry, which was commenced before the publication of these

papers, relates to the action, by salivary and pancreatic amylases, on different varieties of rice (mostly grown in south India), unpolished as well as polished to varying degrees, the action of bran and of bran constituents and, the effect of parboiling on the digestibility of starch.

Experimental

Materials

Rice varieties.—Different pure strains, most of them grown in the Agricultural Research Stations of the Madras Presidency, were used for the studies. Detailed chemical examination of the varieties have been reported in a previous publication [Sadasivan and Sreenivasan, 1938]. Husking, polishing to different degrees and parboiling were all done in the manner outlined in an earlier paper [Sreenivasan *et al.*, 1938].

Enzymes.—Human saliva, diluted with an equal volume of water was used as the source of ptyalin while, a 0·5 per cent solution of trypsin (Pfansteil), which contains amylase, was employed as the source of the pancreatic amylase. The enzyme solutions were centrifuged and filtered before use. When not in use, they were preserved with toluene in an ice chest. The activities of the enzyme preparations used in the following studies were not the same throughout although, for each particular series of experiments, the same preparations were employed.

Method.—Some preliminary experiments were first carried out studying the action of the enzymes on whole grains of rice cooked according to usual procedure. Difficulty was experienced in obtaining homogeneous samples for estimation from the reaction mixture and highly discordant results were being obtained. Accordingly, samples of the different varieties of shelled rice were powdered and passed through sixty-mesh sieve; requisite quantities were then treated with a little water, boiled for about five minutes and then autoclaved for thirty minutes at 15 lbs. pressure. It was thereby ensured that the different samples were gelatinised completely and under comparable conditions. The autoclaved samples were finally made up to a known volume so as to yield in all cases a final suspension of one per cent starch concentration, the starch in the flours being previously estimated according to A. O. A. C. [1930].

The reaction-mixture consisted of 20 c. c. of phosphate buffer having a *p*H of 6·8, 50 c. c. of starch or rice flour suspension, one c. c. of normal sodium chloride solution and 27 c. c. of water. The mixture was placed in a thermostat at 35 $\pm$ 0·1°C. and, when the contents of the flask had acquired the temperature of the bath, 2 c. c. of the enzyme solution was added, thus making a total volume of 100 c. c. Aliquots (usually 20 c.c.) of the reaction mixture were removed immediately after addition of the enzyme and at intervals of five, ten, and fifteen minutes and the reducing sugars estimated by the method of Willstätter and Schudel [1918]. The results were expressed in terms of mg. of maltose in the total volume of the reaction mixture.

Effect of polishing on the digestibility of raw rice

Three varieties (Adt 11, GEB 24, and Co 9) of rice were polished to different degrees and the digestibility of the unpolished and polished specimens by salivary and pancreatic amylases were studied in the manner outlined above (Tables I*a* and I*b*).

TABLE I*a*

Degree of polishing in relation to digestibility of raw rice by ptyalin

Variety Adt 11				Variety GEB 24				Variety Co 9			
Degree of polishing (per cent)	Mg. maltose formed at end of (mins.)			Degree of polishing (per cent)	Mg. maltose formed at end of (mins.)			Degree of polishing (per cent)	Mg. maltose formed at end of (mins.)		
	5	10	15		5	10	15		5	10	15
Unpolished .	155	206	227	Unpolished	227	273	307	Unpolished	244	328	344
6	185	227	244	3	235	319	328	3	252	336	361
10	202	248	260	6	252	332	340	10	239	332	349
16	218	260	277	9	265	353	374	19	235	328	357
22	202	218	269	13	252	336	361				
28	189	218	239	17	260	331	361				

TABLE I*b*

Degree of polishing in relation to digestibility of raw rice by pancreatic amylase

Variety Adt 11				Variety GEB 24				Variety Co 9			
Degree of polishing (per cent)	Mg. maltose formed at end of (mins.)			Degree of polishing (per cent)	Mg. maltose formed at end of (mins.)			Degree of polishing (per cent)	Mg. maltose formed at end of (mins.)		
	5	10	15		5	10	15		5	10	15
Nil	122	197	227	*Nil*	176	269	302	*Nil*	181	260	311
6	134	231	248	3	185	281	315	3	197	281	328
10	147	..	..	6	197	286	315	10	176	269	315
16	143	227	252	9	206	281	256	19	172	248	290
22	126	189	210	13	197	265	281				
28	126	197	239	17	210	290	315				

It may be observed that, in all the varieties, the digestibility increases with polishing, the extent of such increase being different with different varieties. With a very high degree of polishing, there appears, however, to be a slight diminution in the extent of hydrolysis in all the cases. It is probable that, as pointed out later, this is due to the increased viscosity of the cooked rice flour.

More or less similar results are obtained with both the amylases. In view of this and the fact that it was rather difficult to obtain the salivary amylase with nearly the same activity, further studies were carried out with the trypsin preparation alone.

Effect of polishing on the digestibility of parboiled rice

Parboiled rice prepared from two of the varieties (Adt 11 and Co 9) of paddy was polished to three different degrees and the hydrolysis by trypsin-amylase of the different samples was followed as before (Table II).

TABLE II

Degree of polishing in relation to digestibility of parboiled rice by pancreatic amylase

Degree of polishing (per cent)	Variety Adt 11			Variety Co 9		
	Mg. maltose formed at the end of (mins.)			Mg. maltose formed at the end of (mins.)		
	5	10	15	5	10	15
Nil	111	183	221	145	200	247
4	124	205	252	149	217	252
10	136	213	260	158	226	260
15	145	205	247	149	222	273

The results show that, as in raw specimens of rice, the extent of hydrolysis of the rice starch increases with increased polishing while with a high degree of polishing, there is a slight diminution in hydrolysis.

Comparative digestibilities of raw and parboiled rices

In Table III are given the results of comparative digestibilities of raw and parboiled rices from the same varieties (Adt 11 and Co 9), unpolished as well as polished to equal extents (10 per cent).

TABLE III

Digestibility of raw and parboiled rices

Description	Variety Adt 11			Variety Co 9		
	Mg. maltose formed at the end of (mins.)			Mg. maltose formed at the end of (mins.)		
	5	10	15	5	10	15
Raw, unpolished . . .	141	209	247	158	226	235
Parboiled, unpolished .	145	222	269	175	217	256
Raw, polished . . .	171	239	273	217	294	320
Parboiled, polished . .	171	230	264	209	290	337

In the polished specimens, both raw and parboiled rices are digested to about the same extent while with the unpolished specimens, parboiled rice is hydrolysed to a slightly greater extent than the raw one; the difference is however only small. It may be mentioned here that Basu and co-workers [1935, 1936] have reported that parboiling increases the rate of digestion of rice starch in both unpolished and polished specimens.

Viscosity of cooked rice flour

The changes in viscosity of cooked rice flour from raw and parboiled specimens of rice, unpolished and polished, were followed in an Ostwald viscosimeter using the same suspensions that were employed for the above enzymic studies (Tabe IV).

TABLE IV

Changes in viscosity on polishing

	Adt 11—raw rice					
Degree of polishing (per cent) .	0	6	10	16	22	28
Time of flow in viscosimeter (secs.)	122·0	124·5	126·0	129·5	135·0	138·5
	Co 9—parboiled rice					
Degree of polishing (per cent) .	0		4		10	15
Time of flow in viscosimeter (secs.)	115·5		119·6		126·0	129·5

The changes in viscosity as a result of parboiling were also studied (Table V.)

TABLE V

Effect of parboiling on viscosity of cooked flour

Variety	Raw—unpolished	Parboiled—unpolished	Raw—polished 10 per cent	Parboiled—polished 10 per cent
		Time of flow in secs.		
Co 9	127·5	115·3	136·0	126·5
Adt 11	122·0	112·5	126·0	118·3

It may be seen from Tables IV and V that with both raw and parboiled specimens, the viscosity increases steadily with increased polishing. Parboiling decreases the viscosity of cooked rice flour from unpolished as well as polished rice; this may be due to the partial dextrinisation of starch during parboiling.

It has been observed in earlier experiments (Tables I and II) that the enzymic digestibility of starch in rice increases with polishing. Since viscosity also increases with polishing and since starch hydrolysis is known to decrease with increased viscosity of the suspension, it would follow that the effect of polishing on the enzymic digestibility will be even more pronounced than has been indicated in these experiments. The slower rate of hydrolysis observed with a very high degree of polishing may be due to the marked increase in viscosity in these cases.

Digestibility of cooked rice grains

It is believed that parboiled rice is more sustaining than raw rice. It is generally consumed only by the labouring class of people and by those with sedantary occupation. Enzymic hydrolysis however shows a slight increase in the digestibility of rice starch on parboiling. Since in these experiments, only the flour (passed through 60-mesh sieve) was used, it was thought that better indications, comparable with conditions obtaining in practice, would be obtained by using cooked whole grains instead of the flour. Accordingly, 10-grms. lots of raw and parboiled rices were taken as whole grains and cooked under similar conditions; the cooked grains were then shaken with a known volume (200 c.c.) of water. To the mixtures were now added, at 35°C., 5 c.c. of a 0·5 per cent solution of pancreatic amylase. Aliquots (20 c.c.) of the reaction mixture were then withdrawn from time to time and the extent of hydrolysis measured as before by estimation of the reducing sugars formed (Table VI).

TABLE VI

Effect of parboiling on digestibility of cooked whole grains

	Mg. maltose formed in total volume of reaction mixture at the end of:		
	10 mins.	20 mins.	30 mins.
Raw, unpolished . .	85	145	333
Parboiled, unpolished . .	60	111	273

It may be noted that the hydrolysis is slower with parboiled rice than with raw rice. This may be due to the fact that parboiled rice yields on cooking a product which is comparatively stiffer in consistency than raw rice and that as a result, there is lesser penetration of the enzyme solution into the cooked grain in the former case.

That the rate of hydrolysis is determined by the extent of penetration of the enzyme into the grain could be seen from the results (Table VII) of the following experiment. A specimen of (raw, polished) rice was cooked in two lots (10 grms. each) under identical conditions. The cooked grains were, in one set of experiments, dispersed in a large volume of water and *M*/15 phosphate buffer (150 c.c. and 50 c.c. respectively) while in the other, they were added in big (rolled) lumps to the same volume of the mixture in another flask. The mixtures were in each case treated with 2 c.c. of 0·5 per cent solution of trypsin and the rates of digestion measured as before.

TABLE VII

Effect of dispersing on the digestibility of cooked whole grains

	Mg. maltose formed in the total volume of reaction-mixture at the end of:		
	10 mins.	20 mins.	30 mins.
Cooked rice grains (dispersed) .	68	119	350
Cooked rice grains (in lumps) .	34	60	85

In the experiments where the cooked grains were added as lumps, the rate of hydrolysis is scarcely half as much as in the other case where the rice was held thoroughly dispersed in the medium.

The above observations were generally confirmed in some *in vivo* trials carried out by one of the authors [Sreenivasan, *unpublished data*].

Digestibility of different varieties of rice

The different varieties of rice (unpolished as well as polished till free from bran layers) whose chemical composition has been reported in the previous paper [Sadasivan and Sreenivasan, 1938] were compared for their digestibility of starch. In a few cases, parboiled specimens of rice were also employed. The activity of the pancreatic preparation was the same throughout this series of experiments and was checked from time to time with a solution of soluble starch of the same concentration. The results are given in Tables VIII *a* and *b*.

TABLE VIII*a*

Digestibility of rice varieties—raw

Variety*		Mg. maltose formed at the end of			Variety		Mg. maltose formed at the end of		
		5 mins.	10 mins.	15 mins.			5 mins.	10 mins.	15 mins.
Co 9	U	132	158	183	Adt 3	U	124	149	183
	P	183	196	226		P	154	218	213
Adt 7	U	98	124	141	T 134	U	98	136	162
	P	154	218	213		P	136	171	179
Co 5	U	77	94	124	T 111	U	119	124	132
	P	166	205	200		P	162	187	213
T 167	U	107	128	132	T 304	U	55	77	85
	P	136	166	196		P	60	85	90
T 149	U	38	55	55	T 356	U	64	68	94
	P	102	132	158		P	90	136	166
Co 1	U	60	90	107	T 59	U	73	85	98
	P	90	102	128		P	107	136	158
T 150	U	98	136	145	T 523	U	43	72	90
	P	115	179	200		P	98	132	141
Co 10	U	81	102	128	GEB 24	U	90	115	141
	P	102	136	158		P	107	132	162
Adt 11	U	51	81	107	K modan	U	153	171	183
	P	94	145	171		P	149	183	200
D modan	U	107	119	136	B modan	U	119	162	174
	P	132	145	162		P	128	166	179
C modan	U	111	141	158					
	P	158	196	226					

*U = unpolished ; P = polished (for degree of polishing, *vide* Sadasivan and Sreenivasan, 1938, *loc. cit.*)

TABLE VIII*b*

Digestibility of rice varieties—parboiled

Variety*		Mg. maltose formed at the end of			Variety		Mg. maltose formed at the end of		
		5 mins.	10 mins.	15 mins.			5 mins.	10 mins.	15 mins.
T 304	U	60	85	94	T 523	U	43	77	90
	P	64	98	90		P	94	136	145
GEB 24	U	98	128	149	T 167	U	102	119	124
	P	107	136	162		P	145	162	183
Adt 3	U	128	154	192	Adt 7	U	102	128	158
	P	149	209	213		P	149	239	252
T 111	U	128	132	136	D modan	U	115	128	145
	P	162	183	213		P	136	145	166
Co 1	U	64	85	111					
	P	94	107	124					

*U = unpolished ; P = polished.

It may be observed that, as in earlier experiments, polished rice is more readily digested than unpolished rice. In the unpolished specimens, parboiled rice is somewhat more digestible than raw rice. There is however a large amount of individual variation between the different varieties. Some rices digest more quickly than others ; again, in some, the rate of digestion is much slower than in others, while, in a few cases, the differences in digestibility between unpolished and polished specimens are very small. The reasons for such large variations are not known but there is no doubt that among the important factors determining the rate and extent of digestibility of the rices, are the nature of the starch and of the bran constituents.

Inhibiting action of rice bran upon starch hydrolysis

Since the foregoing experiments had shown that the digestibility of rice starch increases with the degree of polishing to which the rice is subjected, at any rate under the ordinary conditions of milling, it appeared probable that the bran and outer polishings of the grain may contain some inhibitor of starch hydrolysis by diastases. Further experiments were carried out with a view to studying the nature of this inhibition by the bran and bran constituents.

Effect of bran extract on starch hydrolysis by pancreatin

A specimen of rice polishings obtained from Co 9 after milling the grain to 20 per cent of its weight was autoclaved with five times its weight of water at 15 lbs. for fifteen minutes ; known volumes of the extract were then added to the suspension of (polished) rice gelatinised as before and their effect on the

rate of hydrolysis by pancreatic amylase studied. The results together with the corresponding values for the unpolished and polished specimens of rice are given in Table IX.

TABLE IX

Effect of bran extract on digestibility of polished rice

Substrate	Mg. maltose formed at the end of		
	5 mins.	10 mins.	15 mins.
Co. 9 unpolished rice (control)	145	192	239
„ polished (20 per cent) (control) . . .	166	213	256
„ polished rice + 2 c.c. of autoclaved bran extract	158	207	247
„ polished rice + 4 c.c. of branex tract. .	149	200	239

The results show that the extract of polishings has a definite inhibitory effect on the digestibility of polished rice and that with 4 c.c. of the extract added to the suspension of polished rice, the extent of hydrolysis of the latter becomes about the same as that of the unpolished rice.

In another set of experiments, 5 per cent extracts of bran obtained from both Adt 11 and Co 9, polished to 10 per cent, were employed. Known amounts of the extracts were added to a 2 per cent solution of Zulkowsky's soluble starch prior to hydrolysis by the enzyme (Table X).

TABLE X

Effect of extracts of polishings on starch hydrolysis

Substrate	Polishings from Adt 11			Polishings from Co 9		
	Mg. maltose formed at the end of			Mg. maltose formed at the end of		
	5 mins.	10 mins.	15 mins.	5 mins.	10 mins.	15 mins.
Sol. starch, 2 per cent (control)	64	128	183	64	128	183
Starch + 2 c.c. extract . .	56	119	175	51	98	158
Starch + 4 c.c. extract . .	51	111	162	43	81	137

As before, there is definite inhibition by the bran extracts, the extent of such inhibition increasing with the amount of extract used. The bran from red rice has a greater inhibiting action than that from white rice.

In Table XI are given the results of the comparative effects of polishings (5 per cent extracts) from raw and parboiled rices.

TABLE XI

Effect of raw and parboiled rice polishings on digestion of starch

Substrate	Polishings from Adt 11			Polishings from Co 9		
	Mg. maltose formed at the end of			Mg. maltose formed at the end of		
	5 mins.	10 mins.	15 mins.	5 mins.	10 mins.	15 mins.
Starch—control . . .	124	222	298	124	222	298
Starch + 5 c.c. of raw rice pol. extract	111	209	277	107	200	247
Starch + 5 c.c. of parboiled rice pol. extract . .	118	213	281	111	209	250

The results show that the effect of the polishings from parboiled rice is definitely less pronounced than that from raw rice.

Action of successive bran layers

The two varieties, Adt 11 and Co 9, both raw and parboiled, were polished to 15 per cent and the polishings collected in three separate fractions, *viz.*, 0 to 5, 5 to 10, and 10 to 15 per cent respectively. From each fraction, a five per cent autoclaved extract was prepared and the inhibitory action of the extracts were studied by adding 5 c.c. of the latter to a 2 per cent solution of Zulkowsky's soluble starch and following the hydrolysis by pancreatin (5 c.c. of 0·5 per cent solution). The results are given in Table XII.

TABLE XII

Inhibiting action on starch hydrolysis by successive bran layers

Substrate	Raw rice						Parboiled rice					
	Adt 11			Co 9			Adt 11			Co 9		
	Mg. maltose formed at the end of (mins.)						Mg. maltose formed at the end of (mins.)					
	5	10	15	5	10	15	5	10	15	5	10	15
Sol. starch—control . .	171	281	341	171	281	341	166	281	333	166	281	333
Sol. starch+5 c.c. extract, fr. I . . .	158	264	316	166	260	311	162	273	324	158	273	316
Sol. starch+5 c.c. extract, fr. II . . .	162	269	324	171	269	328	154	269	324	166	277	324
Sol. starch+5 c.c. extract, fr. III . . .	166	281	333	171	277	333	158	277	320	166	273	320

It may be seen from Table XII that the inhibiting action is most in the first five per cent of polishings, somewhat less in the second fraction and least in the last fraction ; the difference is less pronounced in the case of polishings obtained from parboiled rice where more or less the same effect is obtained with both the first and second fractions of polishings.

Effect of bran constituents

It has been shown elsewhere [Sreenivasan *et al.*, 1938] that, as a result of polishing of the rice grain, a very large part of the fats, minerals and proteins of the latter are removed with the millings. The inhibitory action of rice polishings on amylolytic digestion may therefore be due to one or more of these constituents. With a view to studying further the nature of the inhibitors, the bran was, in one case, freed from fats by extraction with ether while, in another, it was freed as much as possible from the proteins by successive extraction with 5 per cent sodium chloride solution, the bran being later freed from sodium chloride by washing. Extracts (5 per cent) of the fat-free and nitrogen-free polishings were then prepared and their effect on the hydrolysis of starch by pancreatic amylase (1 c.c. of 0·5 per cent) was compared with that of the original polishings (Table XIII).

TABLE XIII

Effect of fat-free and protein-free polishings on starch hydrolysis

Experiment	Polishings from			
	Adt 11		Co. 9	
	Mg. maltose formed at the end of			
	10 mins.	20 mins.	10 mins.	20 mins.
Soluble starch—control . . .	115	192	115	192
Soluble starch + bran extract—control	102	166	94	158
Soluble starch + bran extract (fat-free)	107	183	102	179
Soluble starch + bran extract (protein-free)	107	183	102	175

It would appear from Table XIII that the inhibitory effect of the bran is due in part to the ether extractives and in part to the proteins.

Effect of extracts of tamarind, chillies, etc., and of ghee

In the following experiments, the effect of addition of small quantities of extracts of tamarind, chillies, etc., and of *ghee* and oil on starch hydrolysis by pancreatic amylase (1 c.c. of 0·5 per cent) was studied. In experiments with

ghee or oil, 2 c.c. of 0·6 per cent calcium chloride was added to the substrate, the latter acting as an emulsifier. The results are recorded in Table XIV. Control experiments were carried out in each case immediately after addition of the enzyme.

TABLE XIV

Effect of addition of tamarind, etc., and of ghee *and oil*

Experiment	Mg. maltose formed at the end of	
	10 mins.	20 mins.
Sol. starch—control	171	252
Sol. starch + 2 c.c. 1 per cent green chillies extract	175	252
Sol. starch + 5 c.c. 1 per cent green chillies extract	175	252
Sol. starch + 2 c.c. 1 per cent red chillies extract	171	252
Sol. starch + 5 c.c. 1 per cent red chillies extract	171	247
Sol. starch + 1 c.c. 1 per cent tamarind extract	171	256
Sol. starch + 3 c.c. 1 per cent tamarind extract	175	252
Sol starch + 1 c.c. 1 per cent tamarind extract (dialysed)	175	256
Sol. starch + 3 c.c. 1 per cent tamarind extract (dialysed)	175	256
Sol. starch + 1 c.c. *ghee*	166	247
Sol. starch + 2 c.c. *ghee*	162	239
Sol. starch + 2 c.c. sesame oil	162	239
Sol. starch + 1 c.c. of *M*/1,000 bile salt	183	264

The results show that extracts of chillies or of tamarind have practically no action while *ghee* or oil has a slight inhibitory effect. Bile salt acts as an activator of starch hydrolysis.

DISCUSSION

The rate of starch hydrolysis in the different varieties of rice, both raw and parboiled, increases with increased polishing until, with a very high degree of polishing, there is no further change in digestibility. There is a large amount of individual variation in the digestibility of the different varieties of rice and, generally speaking, the difference in the digestibility of unpolished and polished rice is greater in red rice and in coarser varieties. These latter contain invariably thicker bran coats than the fine varieties [Sreenivasan and Das Gupta, 1936 ; Ramiah, 1936] and hence, the inhibiting action of bran on starch hydrolysis by diastases should be more pronounced in these cases. The effect of bran decreases with successive polishings so that it would appear that the fats, proteins and mineral constituents of the bran which also decrease in concentration with successive polishings may be responsible for the inhibition. Results of experiments show that such inhibition by individual bran constituents on starch hydrolysis is small though definite. Indeed, the difference in digestibility of unpolished and polished specimens is itself so small as to be insignificant in actual practice. Extracts of tamarind and other spices have

practically no effect on starch hydrolysis. but there is no doubt that, in the animal body, they aid digestion, obviously by stimulating the secretion of the digestive ferments.

Comparative trials with raw and parboiled rices have shown that parboiled rice, especially when unpolished, is more quickly digested by the enzymes than raw rice. This is no doubt due to the partial dextrinization of the starch during parboiling. The experiments however relate to the gelatinized flour from the raw and parboiled rices. With the whole grains, it has been observed that parboiled cooked rice digests much slower than raw cooked rice. Parboiled rice stiffens on cooking and standing whereas raw rice cooks to a soft mass. The former requires mastication and, in consequence, causes a liberal flow of saliva. Under such circumstances, it may be more easily digested than raw rice. When bolted in the same manner as raw rice, parboiled rice may, in fact, be difficult to digest on account of the lesser ease of penetration of the enzymes in such cases. It is likely that the better digestibility of stored rice compared to unstored or fresh rice may also be due to the fact that in the former case, the grains (which swell properly on cooking) are better penetrated by the digestive ferments than in the latter where the grains cook to a pasty consistency [Sreenivasan, 1939].

Experiments with single enzymes acting on specific substrates are not by themselves quite sufficient. The human system secretes a variety of enzymes, all of which act on the highly complicated and mixed type of diets that are consumed. Thus, although the fat of rice bran has an inhibitory action on amylases, it may not do so in the human system which also secretes a lipase capable of hydrolysing the bran fat. In a similar manner, the inhibitory action of proteins will be eliminated through the action of pepsin and trypsin. There is great need therefore to work with mixtures of enzymes to obtain a correct indication of the digestibility of whole grains. Further researches along these lines are in progress.

The manner in which the present study on the digestibility of rice varieties has been carried out and the conditions of the digestions are such that the results obtained may not have any direct physiological application; there is no doubt, however, that this study has yielded results of value on the comparative digestibilities of coarse and fine varieties of rice and on the effects of polishing and parboiling on such digestibilities. Further trials on animals or human beings are necessary to confirm these findings.

Summary

1. The digestibility of rice, raw or parboiled, by (pancreatic and salivary) amylases increases with the degree of polishing. Parboiled rice, especially when unpolished, is more easily digested than raw rice.

2. With a very high degree of polishing, there is a slight diminution in the extent of hydrolysis of rice starch. The viscosity of cooked rice flour increases with increased polishing, the rate of such increase being greater with a high degree of polishing.

3. With cooked whole grains of rice, parboiled rice starch is hydrolysed less easily than raw rice starch by pancreatic amylase. The rate of hydrolysis is determined by the extent of penetration of the digestive ferments into the grain.

4. Study of the digestibility of a large number of rice varieties has shown that invariably polished rice is digested more easily than unpolished rice and that parboiling somewhat increases the rate of digestion. There is, however, a large amount of individual variation between the different varieties.

5. Rice bran has a definite inhibitory action on amylases; the extent of such inhibition is greatest with the first layer of polishings and decreases steadily with the subsequent layers. Among the individual bran constituents the ether-extractives and the proteins of the bran exercise definite, though small, inhibitory action on starch hydrolysis by amylases.

Acknowledgments

The authors' thanks are due to Professor V. Subrahmanyan, D.Sc., F.I.C., for his kind interest and to the Government Paddy Specialist, Madras, for the supply of Paddy samples. One of us (A. S.) is also grateful to the Imperial Council of Agricultural Research for a personal grant.

REFERENCES

A. O. A. C. (1930). 'Methods of Analysis'

Basu, K. P. and Mukherjee, S. (1936). *Ind. J. Med. Res* **23**, 777

Basu, K. P. and Sarkar, S. N. (1935). *Ind. J. Med. Res.* **22**, 759

Giri, K. V. (1935). *Science (New York)* **81**, 343

Hammerstein, (1908). *Upsala lakar Forh.* **6**, 471

Lang, (1910). *Zeit. Expt. Path.* **8**, 279

Nagao, (1911). *Zeit. Expt. Path.* **9**, 227

O'Sullivan, J. (1904). *J. Chem. Soc. Trans.* p. 616

Pauletig, (1917). *Zeit. physiol. Chemie*, **100**, 74

Ramiah, K. (1936). *Curr. Sci.* **5**, 215

Reinitzer, (1909). *Zeit. physiol. Chemie*, **61**, 352

Sadasivan, V. and Sreenivasan, A. (1938). *Ind. J. Agric. Sci.* **8**, 807

Sreenivasan, A. (1939). *Ind. J. Agric. Sci.* **9**, 208

Sreenivasan, A. and Das Gupta, H. P. (1936). *Curr. Sci.* **5**, 75

Sreenivasan, A., Subrahmanyan, V. and Das Gupta, H. P. (1938). *Ind. J. Agric. Sci.* **8**, 459

Stone, W. E. (1896). *U. S. Dept. Agric., Stat. Bull.* **34**

Welzmuller, (1921). *Biochem. Zeit.* **125**, 179

Willstätter, R. and Schudel, R. (1918). *Ber. deut. Chem. Ges.* **51**, 780

Wollf and Fernbach, (1907). *Compt. Rend.* **144**, 645

IV. STORAGE CHANGES IN RICE AFTER HARVEST

BY

A. SREENIVASAN, M.A., D.Sc., A.I.C.

Department of Biochemistry, Indian Institute of Science, Bangalore

(Received for publication on 31st December 1938)

I. Introduction

It is well known that freshly harvested rice is not fit for consumption and should be stored for some time before usage. Experience has shown that stored rice cooks better, yields less gruel and digests more easily than the unstored product. Old rice also sells at a higher price and is, besides, more popular.

The problem of storage is of general interest for, most grain and tuber crops need a preliminary period of storage before they can be safely consumed. Thus, wheat acquires ' strength ' on storage and is unsuitable for milling and bread-making before being ' conditioned '. *Ragi* is valued more as a food when stored for some time during which period it is said to improve considerably. Fresh potato, in spite of its agreeable flavour and pleasant taste, causes digestive disorders due, it is believed, to high concentrations of certain salts and should therefore be kept aside for some time after harvest.

Although the need for storage in rice is generally well recognised, yet very little scientific information is available on the precise nature of the changes undergone by the starch and other constituents of the grain during storage. Freshly harvested rice is said to contain a toxic substance of an alkaloidal nature [Hofmeister, 1920] which disappears during storage. It has also been suggested that the absorption of water and consequent swelling takes place through certain small capillaries in the grain and that these capillaries widen during storage, thus allowing of greater imbibition of water [Subbaramiah and Rao, 1937]. According to another explanation, the starch of the grain is assumed to become progressively more complex during storage by a process of slow dehydration [Rao, 1935]. The above conclusions, however, have been drawn only from indirect evidence. In a recent communication, Sahasrabuddhe and Kibe [1935] have proved the existence, in the resting seed, of an active amylohydrolytic enzyme which has the power of attacking both gelatinized and ungelatinized starch of the rice grain and is, to a certain extent, responsible for the greater digestibility of stored rice compared to that of the new rice.

In the present paper, fresh evidence has been presented to show that (*a*) new, unstored rice does not digest as easily as stored rice, mainly because it cooks to a pasty condition and is not therefore penetrated by the digestive ferments as well as the properly swollen grains of the stored rice, and (*b*)

one at least of the factors responsible for the unstored rice cooking to a squashy consistency is the presence in the grain, immediately after harvest, of a fairly active α-amylase which, during storage, passes into a dormant condition [Giri and Sreenivasan, 1937].

II. Cooking quality of unstored and stored rices

The most conspicuous feature of well-stored rice is its capacity for swelling on cooking with water. New rice imbibes very little water and cooks to a paste. On storing, the rice develops a greater capacity for imbibing water. The extent to which this quality improves on storage depends on the method of storage and also the period for which the rice has been stored. In the following experiments, three different varieties of rice were stored as paddy immediately after harvest (1) in loose gunny bags, (2) in bottles loosely plugged with cotton wool and kept (*a*) at room temperature and (*b*) at 0°C. in the ice chest, (3) in tightly stoppered bottles and (4) in sealed flasks (*a*) anaerobically, and (*b*) in presence of carbon dioxide gas. The cooking qualities of the different samples were studied from time to time by measuring the volume expansion during cooking. This was done by displacement of a known volume of water with a definite quantity (usually 10 grms.) of the shelled whole rice grains both before and after cooking under standard and comparable conditions. The measurements were made in a specially improvised dilatometer.

An essentially similar device was employed by the Paddy Specialist at Coimbatore in his studies on the physical analysis and cooking qualities of market samples of rice (*Final Report of Work done at Coimbatore in connection with the Investigations on the Quality of Rice*, 1938, p. 12).

Cooking quality is given by the ratio of the volume of cooked grains to that of the uncooked grains. The results thus obtained are given in Table I.

Table I

Changes in cooking quality on storage

Storage treatment	Variety—Adt 11						Variety—Co 9						Variety—Co 10					
	Period of storage in months																	
	0	1/2	1	2	3	4	0	1/2	1	2	3	4	0	1/2	1	2	3	4
1	1·9	2·2	2·6	3·0	3·6	3·6	1·7	1·9	2·3	2·9	3·6	3·8	1·7	2·1	2·5	3·2	3·4	3·8
2 (*a*)	...	2·1	2·5	3·1	3·5	3·8	...	2·1	2·4	2·9	3·7	3·9	...	2·2	2·6	3·2	3·6	3·8
2 (*b*)	...	1·8	1·9	2·1	2·3	2·7	...	1·6	1·7	1·7	1·9	2·3	...	1·8	1·8	2·0	2·2	2·0
3	...	2·0	2·8	3·3	3·7	4·0	...	1·9	2·5	3·2	3·4	3·7	...	2·1	2·7	3·0	3·6	3·9
4 (*a*)	...	1·9	2·9	3·4	3·8	4·2	...	1·9	2·6	3·4	3·6	3·8	...	2·2	2·8	3·4	3·9	4·1
4 (*b*)	...	2·2	3·0	3·6	4·0	4·3	...	2·0	2·8	3·5	3·9	4·0	...	2·4	2·8	3·5	4·0	4·0

The results show that the cooking quality improves in samples stored in air-tight containers or under anaerobic conditions. Paddy stored in cold chamber does not improve in cooking quality even at the end of four months. In fact, some of the samples taken out after nearly eight months of storage were found to have scarcely improved in their swelling capacities on cooking. Well-stored rice grain swells on cooking to about four times the original volume while the unstored grain does not swell even to twice the original volume. The unstored grain also cooks more rapidly than the stored product. The foregoing observations were confirmed generally by the independent findings made simultaneously by the Paddy Specialist at the Rice Research Station, Coimbatore [K. Ramiah, *private communication*].

It has been observed at Coimbatore that when freshly harvested rice is stored in metallic bins with and without provision for ventilation, the improvement in cooking quality as a result of storage is slighly better in samples stored in ventilated bins than in those kept in closed containers; but this difference is observed only during the early stages and tends to fall off with the period of storage (*Final Report of Work done at Coimbatore*, 1938, p. 2).

It is not clear whether the storage changes have any bearing on the respiratory changes of the grain. The paddy of course continues to live and respire during storage. But, since storage changes are most rapid in properly closed pits or cisterns, it would follow that respiration is not entirely responsible for the attendant transformations.

Measurement of swelling on cooking is an elegant method of determining the extent of storage changes in rice. Another method will be to determine the extent of disintegration as a result of cooking from turbidity measurements or from the amounts of dissolved material. The latter is conveniently determined by precipitation with alcohol, filtering and weighing after drying.

With a view to finding out the extent to which polishing affects the swelling capacity of unstored and stored rices, some trials were carried out with freshly harvested rice and with rice stored for one year, both polished to different known extents [Sreenivasan *et al.*, 1938]. The cooking qualities, measured as before, are given in Table II.

TABLE II

Degree of polishing in relation to cooking quality of unstored and stored rices

Variety	Degree of polishing per cent				
	0	5	8	12	15
Adt 11, new . . .	1·9	2·2	2·4	2·9	2·9
Do. 1 year old . .	4·2	4·4	4·3	4·5	3·8
Co 9, new	1·7	1·9	2·4	2·9	3·2
Do. 1 year old . .	4·0	4·2	4·2	4·4	3·6

It is interesting to note that in the well-stored specimens, the cooking quality increases slightly with polishing until, with a very high degree of polishing, there is a definite decrease. The rice cooks in these cases rapidly and often to a shapeless consistency. With the unstored rice however there is a steady increase in swelling capacity with the extent of polishing. This observation is of especial significance and is again referred to in a later section.

III. Digestibility of cooked unstored and stored rices

It has been shown elsewhere [Sreenivasan and Giri, 1938] that when rice is cooked to a lumpy condition and then subjected to the action of amylohydrolytic enzymes, it does not digest even half as much as when cooked to the usual consistency. This difference in starch hydrolysis is exhibited even by stored rice when the latter is cooked to a paste (by overboiling with excess of water) and to a loose structure. Thus, in the following experiments, a specimen of well-stored (unpolished) rice was cooked in two lots (10 grms. each). In one case, cooking was done to the usual fluffy consistency while in another, the grains were allowed to cook to a squashy condition. The cooked grains were, in either case, added to a mixture of 150 c.c. of water and 50 c.c. of $M/15$ phosphate buffer at pH 6·8. The mixtures were kept in a thermostat at $35° \pm 0·1°$ and, after some time, treated with 2 c.c. of a 0·5 per cent solution of trypsin (Pfansteil). Aliquots of the reaction mixtures were removed immediately after addition of the enzyme and at intervals of 10, 20 and 30 minutes and the reducing sugars estimated by the method of Willstätter and Schudel [1918]. The results, expressed in terms of mg. of maltose in the total volume of the reaction mixture, are given in Table III.

Table III

Effect of cooking to a paste on the digestibility of rice grains

Condition of cooked rice	Mg. maltose formed at the end of		
	10 mins.	20 mins.	30 mins.
Cooked to normal consistency	94	128	218
Cooked to a paste	55	94	122

The properly swollen grains present a greater reactive surface and are hence better penetrated by the digestive ferments than those which are not. A similar phenomenon has been observed by Reddi and Subrahmanyan [1937] who have studied the action of certain moulds on rice cooked to different extents. When the individual grains stand apart, the mould threads

penetrate them easily and convert them into sugar and other products whereas, when the rice is otherwise rendered squashy, the moulds penetrate only partially leaving the hard central portions unattacked.

The above observations were confirmed by some preliminary growth experiments on young rats. Two groups of four rats each were fed, for a period of thirty days, on cooked powdered rice and cooked whole rice respectively from the same variety. The rice was supplemented with small identical quantities of casein, cod-liver oil and salt mixture in both the cases. Weekly average increases in weight are given in Table IV.

TABLE IV

Growth rate with cooked powdered rice and cooked whole rice

Diet	Weeks			
	1	2	3	4
	Average growth rate in grms.			
Cooked whole rice	5·1	4·9	4·4	4·6
Cooked powdered rice	5·1	4·6	4·0	4·3

The growth rate is poorer when the powdered grain is used. This naturally cooks to a paste compared with the whole grain. It would therefore appear that the bad effects of unstored rice are due primarily to its cooking to a shapeless pasty condition and thereby preventing the digestive ferments of the human system from coming into proper contact with the cooked mass. Part of the starch may pass undigested into the lower intestines and thus produce undesirable fermentations and other digestive disorders.

IV. *In vitro* DIGESTIBILITY OF THE STARCHES FROM NEW AND OLD RICES

Pure starches from new and one year old samples of rice from varieties Co 9, Adt 11, GEB 24, and Co 10 [Sadasivan and Sreenivasan, 1938] were prepared as follows :—

The shelled grains were soaked in 20 per cent alcohol and the softened kernels were then ground in a mortar. Enzymic action during the extraction was checked by the addition of $1 \times 10^{-4}N$ iodine [Olsson, 1921]. The pulps thus obtained were extracted thrice with cold, 20 per cent alcohol. The extracts were strained through several thicknesses of cloth and the starch removed by centrifuging and filtering. It was purified by washing in 20 and 80 per cent alcohol as described by Parker [1935]. The starch was washed and centrifuged in 80 per cent alcohol over ten times before it was considered pure. The air-dried specimens were powdered to pass the 100-mesh sieve.

For experiments on the digestibility of the starches by different enzymes, requisite quantities of the starches were treated with a little water, boiled for about five minutes and then autoclaved for thirty minutes at 15 lbs. pressure to ensure that the samples were gelatinized completely and under comparable conditions. Human saliva (1 : 1) and a 0·5 per cent solution of trypsin (Pfansteil) were employed for the studies. The reaction mixtures consisted of 20 c.c. of phosphate buffer (*p*H 6·8), 50 c.c. of one per cent starch suspension, 1 c.c. of normal sodium chloride and 27 c.c. of water. The mixtures were placed in a thermostat at 35°± 0·1° and, when the contents had acquired the temperature of the bath, 2 c.c. of the enzyme solution was added, thus making a total volume of 100 c.c. Aliquots (20 c.c.) of the reaction mixture were removed immediately after addition of the enzyme and at intervals of 5, 10, and 15 minutes and the reducing sugars present estimated by Willstätter and Schudel's [1918] method. The results are recorded in Table V as mg. of maltose formed in the total volume of the reaction mixture.

TABLE V

Digestibility of starch from new and stored rice

Starch from	Salivary Amylase			Pancreatic Amylase		
	Mg. maltose formed at the end of					
	5 mins.	10 mins.	15 mins.	5 mins.	10 mins.	15 mins.
Adt 11, new	159	186	207	122	186	245
Do. old	165	192	228	125	182	245
Co 9, new	196	254	286	141	206	275
Do. old	188	274	296	145	216	290
GEB 24, new	184	242	292	197	265	304
Do. old	184	246	302	194	272	312
Co 10, new	124	196	232	100	136	195
Do. old	118	196	226	122	146	226

It may be seen that though the pure starches from stored rice specimens digest more readily than those from the freshly harvested rices, the differences are not very pronounced. Sahasrabuddhe and Kibe [1935] have reported that the starches from new and old rices do not differ in their physical characteristics although they observe that under the action of hydrochloric acid,

diastase, and pancreatin, the starches from old rices are hydrolysed much quicker than those from new rices. It is possible that in the above experiments the mode of preparation of the starches has obliterated to some extent the differences, if any, in the make up of the starches of new and stored rices. Further work is essential to prove whether this is so or not.

V. Carbohydrate changes during ripening and storage

In view of the fact that carbohydrates predominate in rice, it was considered probable that storage may result in a change in these constituents more than in any other. Accordingly, specimens of rice (finely ground and passed through 60-mesh sieve) from freshly harvested and one year old samples of four different varieties were analysed for their carbohydrate constituents—dextrose, sucrose, other dextrins and polysaccharides, and starch —according to the methods of the A. O. A. C. [1935]. The results are given in Table VI.

Table VI

Carbohydrate constituents in new and stored rices

Variety	Moisture	Dextrose	Sucrose	Dextrins	Starch
	Percentages				
Adt 11, new . . .	13·2	2·12	0·36	1·86	74·8
Do. old . . .	11·8	2·02	0·36	1·34	74·5
Co 9, new	13·4	2·65	0·48	1·56	74·6
Do. old . . .	12·0	2·32	0·43	1·11	74·6
Adt 6, new . . .	11·9	1·85	0·30	1·80	72·2
Do. old . . .	10·4	1·79	0·29	1·26	72·0
Co 10, new	12·6	1·45	0·34	2·05	74·9
Do. old . . .	12·1	1·38	0·36	1·85	73·7

There does not appear to be any marked change in the different carbohydrate constituents as a result of storage.

Storage changes are often considered to be mere continuation of the changes during ripening. To verify this, the changes in carbohydrate constituents were followed from the time the grain began to set. Samples (variety Adt 11) in 2 lb. lots were sent to Bangalore from the Agricultural Research Station, Coimbatore, by Railway Parcel. They were taken from the crop

in the field every fourth day. The time taken for transit varied between two and four days and hence a certain amount of change must have taken place during this period. With the available facilities, this could not be checked. The first sample was taken after the grain had begun to set. In all, nine samples were collected, the last sample representing the fully ripe grain at the harvesting stage. Each sample of paddy was analysed as before for the different carbohydrate constituents immediately after receipt. The harvested sample was also examined at fortnightly intervals during storage. Table VII gives the results obtained.

TABLE VII

Carbohydrate changes in paddy during ripening and storage

Stage of ripening	Moisture	Reducing sugars	Non-reducing sugars	Soluble starch, dextrin, etc.	Starch *
	Percentages				
First	8·7	7·96	0·54	7·42	39·21
Second	8·8	7·01	0·33	6·68	55·42
Third	8·9	7·02	0·38	6·64	58·89
Fourth	8·5	6·45	0·47	5·98	59·95
Fifth	8·7	5·00	0·37	4·63	61·86
Sixth	8·2	4·57	0·25	4·32	62·12
Seventh	8·6	4·59	0·31	4·28	62·19
Eighth	7·1	3·07	0·38	2·69	62·96
Harvest sample	8·1	2·74	0·41	2·33	64·00
Stored—					
(1st fortnight)	8·5	2·68	0·38	2·30	64·15
(2nd fortnight)	8·4	2·71	0·39	2·32	64·26
(3rd fortnight)	8·2	2·72	0·40	2·32	63·85
(6th fortnight)	8·2	2·59	0·35	2·23	65·02

* Determined by taka-diastase hydrolysis followed by Widdowson's [1931] method of sugar estimation.

It may be observed that during ripening there is a steady decrease in reducing sugars, sucrose and dextrin and a rapid increase in starch content. There is however no marked change in these constituents during storage (Table VI).

VI. THE AMYLASE SYSTEM OF THE RICE GRAIN DURING RIPENING AND STORAGE

Rice grains (variety Co 10) were freshly collected from the crop at different stages of ripening. The fresh weights and the moisture contents were determined immediately. The grains were extracted with 13·5 times their

weight of water for twenty-four hours at room temperature. The amount of extractant to be added was always calculated on the dry weights of the grains taken.

The saccharifying power (β-amylase) and the dextrinizing power (α-amylase) of the extracts were determined according to methods outlined by Giri and Sreenivasan [1937, 1938] at *p*H 4·6 (*M*/5 acetic acid-acetate buffer) and 7·0 (*M*/15 phosphate buffer) respectively, these being the *p*H optima of the two amylases [Giri and Sreenivasan, 1936]. The results are presented in Table VIII.

TABLE VIII

Variation in the activities of α- and β- amylases of rice grain during ripening and storage

Stage of grain	Weight of 200 grains grm.	Per cent moisture	β-amylase (at *p*H4·6)		α-amylase (at *p*H7·0)	
			Time of hydrolysis in hours	Activity in mg. maltose in total volume of reaction mixture	Time required in mins. to change colour tone of iodine to	
					Violet	Rose
'Milk' stage	3·937	46·2	1 3 24	8·6 47·0 202·0	30	1200
'Mealy' stage	4·710	37·0	1 3 24	8·6 47·0 200·0	30	1200
Ripe grain	5·992	24·5	1 3 24	8·3 26·0 90·0	1400	..
Stored for one month.	4·740	16·5	1 3 24	7·5 23·0 77·0	1400	..

It will be seen that the activities of both the amylases decrease as ripening advances, while in the resting grain it is almost completely *nil*. It has been shown elsewhere [Giri and Sreenivasan, 1937, 1938] that the amylases in the resting grain are present in a dormant condition and that they can be obtained in a fully active state by extraction with phosphate buffer at *p*H 7·0.

In the following experiments, the activities of the two amylases have been followed for two and a half months during storage, in three varieties of paddy from the time of harvest (Tables IX and X).

TABLE IX

Changes in β-amylase activity during storage

Variety *	Date of harvest	Time of hydrolysis in hrs.	Date of sampling				
			24-6-37	6-7-37	21-7-37	18-8-37	1-9-37
			Activity in mg. maltose in total volume of reaction mixture				
Halublu (I)	12th June 1937	1	11·4	9·2	6·8	4·8	4·3
		3	39·2	18·6	9·8	11·9	8·9
		24	92·6	74·2	55·0	44·6	33·8
Kempasanna (II)	8th June 1937	1	10·8	7·6	4·4	8·6	*Nil*
		3	38·6	23·9	14·6	12·2	8·8
		24	74·2	94·6	74·6	65·7	33·6
Three month crop (III)	16th June 1937	1	10·2	4·8	*Nil.*	4·3	*Nil*
		3	29·6	16·2	8·5	9·8	4·8
		24	77·0	68·4	42·4	38·6	26·0

* These varieties were obtained from the Hebbal Agricultural Farm through the courtesy of the Superintendent.

TABLE X

Changes in α-amylase activity during storage

Time in mins.	Date of sampling														
	24-6-1937			6-7-1937			21-7-1937			18-8-1937			1-9-1937		
Variety	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
	Colour tone with $N/200$ iodine solution														
15 . .	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
30 . .	BV	BV	B	..	..	..	..	..	..	..	..	..	..	..	..
45 . .	BV	BV	BV	BV	B	B	BV	B	B	..	..	..	..	..	..
60 . .	..	..	..	BV	BV	BV	BV	BV	B	BV	B	B	..	..	..
90 . .	..	..	..	..	..	..	BV	BV	BV	..	..	..	BV	B	B
120 . .	V	BV	BV	..	..	..	..	..	..	BV	BV	B	BV	B	B

B = Blue; BV = Blue-violet; V = Violet.

The changes in cooking quality as measured by the extent of volume increase during cooking for the three varieties and at the corresponding dates of sampling are set forth in Table XI.

TABLE XI

Changes in cooking quality during storage

Variety	Date of sampling				
	June 24th	July 6th	July 21st	Aug. 18th	Sept. 1st
Halublu	2·4	2·9	3·3	3·4	3·4
Kempasanna . . .	2·6	3·2	3·6	3·9	4·1
Three month crop . .	2·0	3·0	3·9	4·1	4·2

It may be seen that as before (with Co 10, *vide* Table VIII) the activities of the amylases decrease with the period of storage. There is a close similarity observed between the α-amylase activity and the extent of swelling during cooking. Thus with the Halublu variety, the dextrinizing enzyme does not become as rapidly inactive with storage as with the other two varieties. Likewise, the improvement in cooking quality is less pronounced in this than in the other two varieties.

VII. Discussion and general conclusions

The symptoms attending the consumption of unstored rice are essentially those of poor digestion. One feels heavy shortly after eating such rice and sometimes complains of stomach pains and occasionally even of diarrhoea. These only suggest that unstored rice is slow to digest ; that a part of it passes into the smaller intestines and colon, and, being acted there by the putrefactive bacteria, causes fermentation followed by the usual symptoms of diarrhoea.

It has been shown above that although new rice is really more cooked than the stored grain, the digestive juices do, nevertheless, find it difficult to attack it with the same facility as the latter. Cooked, unstored rice being lumpy and held together by the adhering gruel, presents less reactive surface to the action of the ferments.

A further observation which is of some practical significance in connection with the ill-effects of unstored rice is that it is generally consumed in larger quantities. This is due to the fact that it occupies much less volume than cooked stored rice so that a proportionately larger quantity is eaten before the consumer gets the desired sense of satisfaction.

This raises the question as to why unstored rice cooks always to a paste. It is interesting to note that there is present in the grain, immediately after harvest, an enzyme capable of liquefying or dextrinizing the starch and that this enzyme becomes dormant and inactive when the grain is stored for some time. This α-amylase may be responsible for fresh rice cooking to a squashy condition. The enzyme is very resistant to heat and is not inactivated except on heating to a temperature of about 55° to 60°C. [Yamagishi, 1937]. Rice is usually cooked by adding the grains to water kept nearly boiling. This process of cooking takes a certain time and the grains do not attain the temperature of boiling water until after some time. During this period of cooking, there is obviously, considerable action by the α-amylase on the starch resulting in partial liquefaction.

That it is probably so has been tested in a number of ways. Thus, the pastiness of fresh rice on cooking was found to be greater when this rice was added initially to water and the mixture brought to boil. In this case, the enzyme acts on the starch evidently for a greater length of time before getting inactivated. Again, when fresh rice was dry-heated at about 60° for two or three hours and then cooked, its swelling capacity was greatly improved and compared well with that of the well-stored product. This is due no doubt to the inactivation of the α-amylase during the drying. Mere storage at ordinary temperatures for extended periods also results in such inactivation, but, whereas here the enzyme only passes into a dormant condition [Giri and Sreenivasan, 1937], it is completely destroyed in the former case.

Study of the distribution of the liquefying and saccharifying components of the amylase system in the different parts of the rice grain has shown that these—especially, the liquefying amylase—are mostly localised in the peripheral layers and the germ [Giri and Sreenivasan, 1938 ; Yamagishi, 1937]. These are the very fractions that are lost during the polishing of rice. Hence it is—probably—that the swelling capactity on cooking of unstored rice increases with polishing (Table II).

It is well known that parboiling is one of the potent methods by which freshly harvested rice can be made to swell to its full and proper consistency and thus rendered fit for consumption. This would suggest that the process of heating during parboiling destroys the liquefying amylase. Parboiled rice has a translucent appearance because of the fact that there is partial dextrinization of the starch during parboiling [Sreenivasan *et al.*, 1938]. This might itself have been brought about by the enzyme before destruction.

Quite apart from the inactivation of the starch-splitting ferment, there may be other factors also responsible for the storage changes in rice, which are not properly understood.

A great deal of useful information has, however, been gathered with regard to the methods of storage and means of hastening the same. Thus, as already mentioned, the storage changes take place most rapidly in closed

containers well insulated with adequate plastering against loss of heat by radiation. Increased temperature, up to a point, favours storage changes. On the other hand, a lowered temperature causes distinct retardation.

Short-duration varieties need longer periods of storage than long-duration ones. A few varieties, like GEB 24, become fit for consumption almost immediately after harvest [K. Ramiah, *private communication*]. Paddy harvested in the warm and dry months requires considerably lesser time for storage than that obtained during cold and wet weather. Harvesting practices essentially affect the extent of storage changes necessary and, hence, the time of storage is often a relative feature not entirely dependent upon the variety involved [Srinivasan, 1934].

Although storage by itself involves very little expense, yet neither the producer nor the consumer will like to have the rice on hand for such a long period as it now requires. Quickening of the related changes is highly desirable and it will therefore be of some practical interest to devise improved methods of storage.

Drying in a current of air at 50° to 60° for a few hours is indeed an efficient method of hastening storage changes, but the great drawback in practice of such a method is the development of 'sun-cracks' in the grain, due to partial dehydration at various points in the surface. The cracks disappear during cooking but they do, nevertheless, affect the shelling and milling qualities of the grain adversely.

The observation, that the difference in digestibility between unstored and stored rice is not exhibited to the same degree by the pure starches prepared out of them (Table V), would suggest that there is probably not much difference in the make-up of the starch in the two cases. Further work is essential to elucidate the precise nature of the starch and other constituents in rice and their changes during storage. In some experiments, the problem of storage in rice was studied in relation to (*a*) the limit of saccharification of the starch in the grain, and (*b*) the extent of fractional liquefaction of starch at different temperatures [Warth and Darabsett, 1914]. The bearing of these results [Sreenivasan and Giri, *unpublished data*] on the quality of unstored and stored rices, as also the changes in the starch components of the grain during storage, are being investigated and will be reported in a future communication.

Study of the degradation of starch by different amylases would yield results that may throw light on certain features of its constitution that have not hitherto been approachable by purely chemical means [Hanes, 1937]. A further line of investigation would consist in measuring quantitatively the change of colour with iodine and establishing the relationship between this and the stage and type of degradation that has been induced.

VIII. Summary

1. Well-stored rice grains swell on cooking to about four times the original volume while the unstored grains from freshly harvested paddy scarcely swell to twice the original volume.

2. Increased temperature and reduced air supply during storage quickly improve cooking quality of unstored rice. Paddy stored in cold chamber does not improve much in cooking quality even after several months.

3. Polished, unstored rice swells on cooking to a greater extent than the corresponding unpolished specimen; polishing also improves cooking quality of stored rice, though with a high degree of polishing swelling capacity decreases.

4. *In vitro* experiments have shown that when rice is cooked to a paste, it does not digest even half as much as when cooked to the usual fluffy consistency. The properly swollen grains present a greater reactive surface and are better penetrated by the digestive ferments than those which are not. Growth rate in young rats fed on cooked powdered rice is also less compared with that on rats fed on cooked whole rice.

5. There is only a small though definite difference in the *in vitro* digestibilities of the pure starches prepared out of freshly harvested and well-stored specimens of rice. The latter is hydrolysed more easily than the former.

6. During the ripening of the rice grain, there is a steady decrease in the reducing and non-reducing sugars and dextrin followed by a rapid increase in the starch content. During storage, there is no marked change in the amounts of the various carbohydrate constituents of the grain.

7. The resting seed contains both a liquefying (α) and a saccharifying (β) amylase. The activity of the amylases is greatest at the 'milk stage' of formation of the grain and decreases with the advance in ripening; there is a further decrease in their activity during the storage of the rice grain. In the well-stored grain, the amylases exist in a dormant condition and cannot be extracted with water. There is a close parallelism between the decreased activity of the α-amylase during storage and the improvement in swelling capacity on cooking.

8. The bearing of these observations on the mechanism of storage changes in rice and the nature of further work is discussed. It is concluded that the pastiness on cooking, of unstored rice may, to a certain extent, be due to the starch liquefying ferment (α-amylase) of the seed; the inactivation of this enzyme during storage is followed by increased swelling capacity on cooking.

IX. Acknowledgments

The author's thanks are due to Prof. V. Subrahmanyan, D.Sc., F.I.C., F.N.I., for his continued interest in the progress of this work; to Mr. H. P. Das Gupta, M.Sc., who was responsible for the determination of changes in

carbohydrate constituents during the ripening of the rice grain; to Dr. K. V. Giri, M.Sc., D.Sc., A.I.I.Sc., for assistance in the studies on the amylolytic changes during ripening and storage; and to the Paddy Specialist, Government of Madras, for the supply of the paddy samples.

Thanks are also due to the Imperial Council of Agricultural Research for a personal grant.

REFERENCES

A. O. A. C. (1935). 'Methods of Analysis,' p. 341

Giri, K. V., and Sreenivasan, A. (1936). *Nature* **138,** 406

——————————— (1937). *Biochem. Zeit.* **289,** 155

——————————— (1938). *Biochem. Zeit.* **296,** 428

Hanes, C. S. (1937). *New Phytol.* **36,** 189

Hofmeister, F. (1920). *Biochem. Zeit.* **103,** 218

Olsson, U. (1921). *Zeit. Physiol. Chemie* **117,** 91

Parker, M. W. (1935). *Plant Physiol.* **10,** 713

Rao, B. S. (1935). 'Symposium on Colloids,' Indian Academy of Sciences, Dec. 1935

Reddi, K. R., and Subrahmanyan, V. (1937). *Trans. Natl. Inst. Sci.* (*India*) **1,** No. 11

adasivan, V., and Sreenivasan, A. (1938). *Ind. J. Agric. Sci.* **8,** 807

Sahasrabuddhe, D. L., and Kibe, M. M. (1935). *Ind. J. Agric. Sci.* **5,** 1

Sreenivasan, A., and Giri, K. V. (1939). *Ind. J. Agric. Sci.* **9,** 193

——————— Subrahmanyan, V., and Das Gupta, H. P. (1938). *Ind. J. Agric. Sci.* **8,** 459

Srinivasan, C. R. (1934). 'Rice Production and Trade in the Madras Presidency'; Government Press, Madras

Subbaramiah, K., and Rao, B. S. (1937). *Proc. Ind. Acad. Sci.* **6B,** 36.

Warth, F. J., and Darabsett, D. B. (1914). *Mem. Dept. Agric. Ind.* (*Chem. Ser.*) **3,** No. 5

Widdowson, E. M. (1931). *Biochem. J.* **25,** 863

Willstätter, R., and Schudel, R. (1918). *Ber. d. Deutsch. Chem. Ges.* **51,** 780

Yamagishi, G. (1937). *J. Agric. Chem. Soc.* (*Japan*) **13,** 1268; 1284

ENTOMOLOGICAL INVESTIGATIONS ON THE LEAF-CURL DISEASE OF TOBACCO IN NORTHERN INDIA

III. THE TRANSMISSION OF LEAF-CURL BY WHITE-FLY, *BEMISIA GOSSYPIPERDA*, TO TOBACCO, SANN-HEMP AND A NEW ALTERNATE HOST OF THE LEAF-CURL VIRUS

BY

HEM SINGH PRUTHI, M.Sc., PH.D. (CANTAB.), F.N.I.

Imperial Entomologist

AND

C. K. SAMUEL, B.Sc.

Assistant Entomologist, Tobacco Research Scheme, Imperial Agricultural Research Institute, New Delhi*

(Received for publication on 22nd June 1938)

(With Plates VII-X and one text-figure)

CONTENTS

		Page
I.	INTRODUCTION	224
II.	TECHNIQUE, MATERIAL, ETC.	224
III.	TRANSMISSION OF THE DISEASE FROM SANN-HEMP TO TOBACCO	226
IV.	TRANSMISSION OF THE DISEASE FROM TOBACCO TO SANN-HEMP	229
V.	TRANSMISSION OF THE DISEASE FROM SANN-HEMP TO SANN-HEMP	231
VI.	TRANSMISSION OF THE DISEASE FROM TOBACCO TO TOBACCO	232
VII.	*AGERATUM CONYZOIDES*, A NEW IMPORTANT HOST OF THE TOBACCO LEAF-CURL VIRUS	235
VIII.	TRANSMISSION OF THE DISEASE FROM TOBACCO TO *AGERATUM CONYZOIDES*	236
IX.	TRANSMISSION OF THE DISEASE FROM *AGERATUM CONYZOIDES* TO SANN-HEMP AND BACK TO THE WEED	236
X.	TRANSMISSION OF THE VIRUS FROM DISEASED TO HEALTHY *AGERATUM* WEED	237
XI.	NUMBER OF WHITE-FLIES AND THE PERIOD OF THEIR FEEDING ON DISEASED SOURCE NECESSARY FOR THE TRANSMISSION OF LEAF-CURL AND THE INCUBATION PERIOD OF THE DISEASE INSIDE THE PLANT	237
XII.	OTHER PLANT HOSTS OF WHITE-FLY	243
XIII.	DISCUSSION AND CONCLUSIONS	244
XIV.	ACKNOWLEDGMENTS	246
XV.	REFERENCES	246

*The scheme is financed by the Imperial Council of Agricultural Research to whom the authors are indebted for the financial aid.

I. Introduction

In the second part of our investigations on tobacco leaf-curl [Pruthi and Samuel, 1937] we stated that sann-hemp at Pusa also suffers from leaf-curl and described some transmission experiments performed during 1935 which showed that white-fly, *Bemisia gossypiperda* M. & L., could transmit the disease from sann-hemp to healthy tobacco. During the last two tobacco seasons (1936-37 and 1937-38) we have performed a large number of transmission experiments with white-fly to confirm our previous conclusions referred to above and to ascertain if this insect could also transmit the disease from tobacco back to healthy sann-hemp and from tobacco to tobacco and sann-hemp to sann-hemp. Furthermore, during the course of our work we have observed that *Ageratum conyzoides*, which is a very common weed at Pusa and which also suffers from a leaf-curl disease, serves as a food plant for the white-fly. We have experimented with this weed to determine if this plant, like sann-hemp, is an alternate host of the tobacco leaf-curl virus. Several large series of experiments on the lines indicated above have been performed at different times of the year on tobacco and other hosts of different ages to determine the season when the white-fly is most viruliferous and the ages of various hosts when they most readily get infected and develop the disease. Some very interesting results have been obtained which are reported in the present paper.

II. Technique, material, etc.

In our previous investigations [Pruthi and Samuel, 1937] we used cellophane tubes for encasing the white-fly on the diseased and healthy plants for feeding. As whole leaves had to be enclosed inside the tubes, the latter had necessarily to be very large. It was noticed that the white-flies generally remained in the corners and folds of the crumpled portions of the tubes instead of sitting and feeding on the enclosed leaves. As the longevity of the white-fly in captivity is rather short, it seemed probable that the encased individuals did not feed on the diseased plants at all or, if they did so, they did not feed for sufficient time on the healthy plants on which they were transferred. Furthermore, cellophane has the very serious draw-back in that with slight changes in weather conditions it readily expands and shrinks and consequently becomes very brittle. Therefore in the experiments carried out during the last two years and reported in the following pages, the technique of feeding the white-fly was considerably modified. Instead of a large cellophane tube for encasing an entire leaf, a small glass tube (1 in. $\times \frac{1}{4}$ in.) containing the individuals was fixed on a portion of the leaf by means of a light wire spring which clasped the tube and leaf together as shown in Fig. 1. To avoid injury to the leaf, the end of the spring which rested on it was flattened into a disc and was separated from the leaf surface by means of a felt piece. The white-fly generally feeds on the lower surface of the leaf, therefore, the tubes were also fixed on that surface. This as well as the fact that the white-fly always flies up when put in a tube ensured the settling of the individuals on the portion of the leaf covering the open end of the tube.

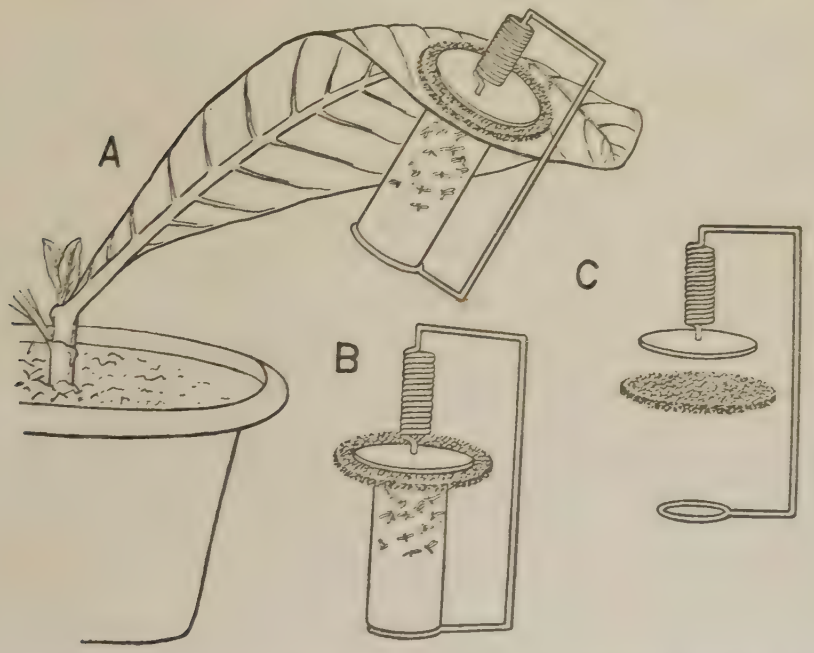


FIG. 1. Diagrammatic illustration of the technique of feeding white-flies on tobacco leaf—*A*. The whole mechanism *in situ*; *B*. The spring and the tube containing the individuals; *C*. The spring showing the felt piece separated from the disc.

The period for which the white-flies were kept on the diseased plants before their transfer to healthy hosts is referred to as the 'feeding period' in the following pages. This, however, is not a very accurate term as the individuals did not necessarily feed for all the time when encased on the leaves After the individuals had been kept on diseased leaves for a definite period. they were transferred to healthy plants on which they were allowed to remain till they died. This was done to ensure their feeding on these plants for the maximum possible period as the percentage infection is known to increase with increased feeding time of the vector on the healthy plant. No record was kept of the period for which the white-flies lived on healthy plants. The total longevity of the white-fly while encased on diseased and healthy, plants varied between three and seven days during August-December. When kept on diseased leaves throughout its life, it did not live for more than about four days. If kept on healthy plants throughout, it remained alive for six to ten days. These observations do not support the statement of Smith [1931] that an infective insect does not present any visible variation from a normal individual.

Tobacco seedlings meant for infection purposes and the controls were raised under insect-proof conditions and were transplanted in pots containing sterilized soil which were kept covered with muslin bags throughout the life

of the plants. Similarly sann-hemp and *Ageratum* plants were kept under insect-proof conditions.

The work was carried out at Pusa (Bihar) and most of the transmission experiments were performed during August-December, the usual period of active growth of tobacco crop in Bihar, as it is recognized [Smith, 1935] that it is difficult to infect mature plants with virus diseases.

The infected plants were kept under observation up to the middle of the following April for noting the appearance of the disease. By this date all the tobacco plants both in pots and in the field had flowered and formed capsules.

The variety of tobacco under investigation was H 142, which is the most common cigarette tobacco grown at the Botanical Sub-station at Pusa.

The symptoms of the various types of leaf-curl of tobacco have not been described in the following pages, as Pal and Tandon [1937] have already published a detailed account thereof. We may, however, state that almost all the types undergo some change with the growth of the tobacco plant and towards the end of the season show some symptoms which have been described by Pal and Tandon as characteristic of *X* type. It may also be added that *A* type always starts as *X* type and in the course of two or three weeks acquires the typical characteristic of *A* type, therefore the two types cannot be distinguished from each other for sometime except that the plants which are going to develop *A* type have more severe symptoms of *X* type than those which are going to remain of *X* type. We have observed the same changes in the field and, therefore, venture to suggest that the descriptions of various types given by Pal and Tandon need some modifications in reference to the changes which the types undergo during different seasons of the year.

III. Transmission of the disease from sann-hemp to tobacco

Five series of experiments were performed between September and the middle of December 1936 on tobacco seedlings, eight to sixteen weeks old. The diseased sann-hemp used as source for these experiments had been sown in the field about the 1st July. The detailed particulars of the experiments are given in Table I-A.

In the first series, seven experiments were performed between the 6th and 12th September, when sann-hemp and tobacco under experiment were both about eight weeks old. Two to five specimens of the white-fly were kept on a diseased sann-hemp plant for twenty-four to forty-eight hours before they were transferred to a healthy tobacco seedling. None of the seven infected seedlings developed the disease.

In the second series, eight experiments were performed between the 20th and 29th September with both sann-hemp and tobacco ten to twelve weeks old. Two to twelve white-flies were kept on diseased sann-hemp for eighteen to fifty-three hours before they were transferred to a healthy tobacco seedling. After about thirty-six days one seedling (No. 1) out of eight developed leaf-curl of *A* type.

The third series consisted of ten experiments performed in the beginning of November with tobacco and diseased sann-hemp being seven and seventeen weeks old respectively. Nine to twenty-six individuals were used and they were kept for 18½ hours on the diseased source. Five out of ten infected tobacco seedlings (Nos. 1 and 7—10) developed leaf-curl. Three seedlings developed leaf curl of *A* type, and in the other two the disease was of *X* type. Whereas in the seedlings which developed *A* type, the disease was evident in about seventeen to nineteen days only, in those which developed *X* type it took as many as seventy-four and eighty-four days to appear.

At about the same time (beginning of November), seven more experiments were performed (series IV) on sann-hemp and tobacco, both seventeen weeks old. Seven to seventeen white-flies which had fed for twenty-four hours on diseased sann-hemp were put on a healthy tobacco seedling. None of the seven infected plants developed leaf-curl.

Again about the middle of December when the diseased sann-hemp was about twenty-two weeks old, ten experiments were performed on about eight weeks old tobacco seedlings (series V). Two seedlings developed leaf-curl in thirty-seven and sixty-three days respectively. The disease was of *X* type.

All the controls in the five series of experiments described above remained free from leaf-curl.

From the foregoing it will be evident that in 1936, as in the previous year, the white-fly was found capable of transmitting the leaf-curl virus from diseased sann-hemp to healthy tobacco which consequently developed disease of *A* type in most cases. It will be further noticed that the highest percentage of positive results (50 per cent) was obtained if the tobacco seedlings were about seven weeks old at the time of infection, as was the case in series III. Experiments performed at the same time and under the same conditions as those in series III but on seventeen weeks old tobacco gave much fewer positive results.

In the course of experiments carried out in 1936 and described above it was observed that with diseased sann-hemp as the source, some of the infected tobacco seedlings developed *A* type while others developed *X* type. In 1937 the symptoms of leaf-curl of sann-hemp were carefully studied before experiments were performed. Two distinct types of the disease could be made out. In one the leaf blades were simply curled but not puckered and there was only a slight thickening and greening of the veins. The second type was much more severe and was characterised by distinct puckering and twisting of leaf blades. In Plate VII, figs. 1 and 2 the two types are illustrated. They are designated as S.V.1 and S.V.2 in the following pages.

Five series of experiments were performed during 1937, two using S.V.1 type and three with S.V.2 type as source. The particulars of experiments

under various series are given in detail in Table I-B, and are briefly summarized in the following statement :—

Main results of transmission experiments from sann-hemp to tobacco, during 1937 (detailed particulars in Table I-B)

Series	Time of experiment	Age of tobacco infected (weeks).	No. of white-flies and the period for which they fed on diseased sann-hemp	Type of disease used as source	No. of plants infected	No. of plants which developed disease and their type	Remarks
			No. / hrs.				
I	9th-11th Sept. 1937	8	6—14 / 29	S.V.1	12	10, *X* ; 2, *D* ?	One *D* recovered
	Do. . .	8	7—14 / 24	S.V.1	28	2, *X* ; 2, *X* ?	Three recovered
II	27th Sept. to 1st Oct. 1937	7	9—27 / 20—48	S.V.1	27	22, *X* .	
III	27th-29th Sept. 1937	7	10—27 / 20—48	S.V.2	3	2, *A*	
IV	31st Oct. to 2nd Nov. 1937	7	5—25 / 24—29	S.V.2	27	15, *A*	
V	25th-28th Nov. 1937	6	3—7 / 24	S.V.2	25	1, *A*	

N. B.—None of the controls developed leaf-curl.

From the foregoing it will be evident that the white-fly can transmit both types of leaf-curl from sann-hemp to tobacco, S.V.1 and S.V.2 types giving rise to *X* and *A* types respectively in tobacco. Photographs of the tobacco plants in which *A* type was produced by experiment are reproduced in Plate VIII, figs. 1*a-h*. Figs. *j-k* are of the controls.

The least amount of time tried for which the white-fly was kept on the diseased source before it was capable of transmitting the disease was twenty hours. The maximum number of positive results was obtained from September up to the middle of November, if the tobacco seedlings at the time of infection

were six to eight weeks old. During the second half of November and December, even if the infected tobacco was of this age, the percentage of positive results was smaller.

Thus the results obtained in 1936 and 1937 clearly confirm those of 1935 in that sann-hemp is an important host of some types of tobacco leaf-curl and that the white-fly, *Bemisia gossypiperda*, can easily transmit the disease from sann-hemp to tobacco.

IV. Transmission of the disease from tobacco to sann-hemp

It has been shown above that the white-fly can easily transmit leaf-curl of both types (S.V.1 and S.V.2) from sann-hemp to tobacco. During 1936, four series of experiments were performed to ascertain if this insect could transmit the disease in the opposite direction, *viz.*, from tobacco to sann-hemp. The detailed particulars of the experiments are given in Table II-A. One to thirty white-flies were kept on diseased tobacco of *A* and other types for eight to forty-eight hours and were then transferred to healthy sann-hemp plants, nine to sixteen weeks old. Thirty-two plants were thus infected between the 12th of September and 6th of November. The sann-hemp seedlings under inoculation had been sown in the beginning of July. Only three infected plants (series III, No. 1-3) showed some reaction but none developed typical leaf-curl.

The incidence of leaf-curl in sann-hemp crops at Pusa is very high during the last week of July and whole of August. For the disease which appears at this time, the infection probably takes place some time before the middle of July. The failure to transmit the disease from tobacco to sann-hemp in the experiments referred to above seemed to be probably on account of the fact that sann-hemp at the time of experiments (September-November) had passed the growth stage at which it was susceptible to the infection. We could not perform any transmission experiments during July as no diseased tobacco for using as source was available before September. However, we kept by careful tending some diseased tobacco plants of 1936 for performing experiments on sann-hemp during July-August of the next year (1937), which are described below.

With the diseased tobacco plants of 1936 as source, four series of experiments were performed during 1937, full particulars of which are given in Table II-B, and the results are briefly summarized in the following statement :—

Main results of transmission experiments from tobacco to sann-hemp during 1937 (detailed particulars in Table II-B)

Series	Time of experiment	Age of sann-hemp infected (weeks)	No. of white-flies and the period for which they fed on diseased tobacco	Type of disease used as source	No. of plants infected	No. of plants which developed disease and their type	Remarks
			No. / hrs.				
I	16th-17th July 1937	2½	2—6 / 4—16	*X*	17	1, S.V.1	
II	18th-23rd July 1937	2½—3	3—7 / 8—13	*C*	5	1, S.V.1	
	Do. . .	,,	,,	*A*	13	1, S.V.2	
	Do. . .	,,	,,	*X*	4	2, S.V.1	
III	23rd-29th July 1937	3—4	1—9 / 6—18	*C*	8	3, S.V.1	
	Do. . .	,,	,,	*A*	10	..	
	Do. . .	,,	,,	*D*	3	..	
	Do. . .	,,	,,	X	4	..	
IV	27th-31st Aug. 1937	8	2—25 / 6—17	*C*	3	1, S.V.1	
	Do. . .	,,	,,	*C*+*D*	6	1, S.V.1	
	Do. . .	,,	,,	*A*	13	6, S.V.2	
	Do. . .	,,	,,	*X*	2	..	

From the above statement it will be clear that white-fly could transmit leaf-curl from tobacco back to sann-hemp, though the proportion of positive results (about 18 per cent) was much smaller than in the case of the transmission of the disease from sann-hemp to tobacco. The disease generally appeared three to five months after infection. A typical sann-hemp plant in which leaf-curl (S.V.2 type) was produced by experiment is illustrated in Plate VII, figs. 4 and 4*a*.

It will be observed that when diseased tobacco used as source was of *A* type, sann-hemp developed S.V.2 type and when it was *X* or *C* type, it developed S.V.1 type.

From the review of the results obtained in 1936 and 1937 it is evident that sann-hemp can get the infection from tobacco generally during July and August only, and that too in a small number of cases. As diseased tobacco is not in the field in North Bihar before the end of September or October, there is very little chance of this crop acting as a source of infection for sann-hemp.

V. Transmission of the disease from sann-hemp to sann-hemp

In 1936 two series of experiments were performed to ascertain if the white-fly could transmit the virus from diseased sann-hemp to healthy sann-hemp. The particulars of the experiment are given in detail in Table III-A. The first series consisted of twenty-four experiments in which four to twelve specimens of the white-fly kept on a diseased plant for twenty-four hours (between the 8th-13th October) were put on a healthy plant which was thirteen to fourteen weeks old at that time. Four out of twenty-four plants thus infected (Nos. 1, 7, 8 and 19) showed leaf-curl almost similar to that of the source. The disease took 57, 29, 27 and 136 days respectively to appear. In all the cases the side shoots of the plants also showed leaf-curl. The second series consisted of ten experiments in which eleven to twenty-seven white-flies which had been allowed to feed on diseased plants for nineteen hours on 4-5th November were introduced on about four months old healthy plants. One plant (No. 9) showed leaf-curl on 6th December. Its side shoots were also diseased.

Thus the white-fly could transmit leaf-curl from diseased to healthy sann-hemp, though the percentage of successful transmissions was rather low (10-16 per cent).

In 1937 two series of experiments were again performed on the above lines to verify the results obtained in the previous year. The particulars of the experiments are set forth in Table III-B. In the first series, consisting of eight experiments, type S.V.1 was used as source. Infections were done in the first week of September when the infected plants were about nine weeks old. Two to five white-flies were kept on a diseased plant for twenty hours before they were transferred to a healthy plant. Two out of eight infected plants developed leaf-curl after seventy-one and seventy-nine days respectively.

In the second series, consisting of nine experiments, diseased plants of type S.V.2 were used as source. Other conditions, *e.g.*, the number of white-flies used, the period for which they were kept on the diseased plants, the age of the infected plants, etc., were the same as in series I above. Two out of nine infected plants developed leaf-curl similar to that of the source after sixty-seven to seventy-two days.

Thus the results of two years' experiments show that white-fly can transmit leaf-curl of both types from sann-hemp to sann-hemp, but the percentage of positive transmissions (10-20 per cent) is small.

VI. Transmission of the disease from tobacco to tobacco

To ascertain the role played by the white-fly in the transmission of leaf-curl of various types from diseased to healthy tobacco plants, seven large series of experiments were performed at different times during 1936, the particulars of which are given in detail in Table IV-A and the results are summarized briefly in the following statement :—

Main results of the experiments performed for the transmission of leaf-curl from diseased to healthy tobacco during 1936 (detailed particulars in Table IV-A)

Series	Time of experiment	Age of tobacco infected. (weeks)	No. of white-flies and period for which they were fed on diseased plant	Type of disease used as source	No. of plants infected	No. of plants which developed the disease of various types	Remarks
1	2	3	4	5	6	7	8
i	14th-29th Sept. 1936	9—11	No. / hrs. 5 / 19	*B*	3	*Nil*	...
			3—12 / 16—48	*C*	8	1, *C*	
			1—3 / 16—17	*X*	6	1, *X*	
ii	8th-15th Oct. 1936	12—13	3—5 / 8—9	*A*	5	1, *X*	The source was not pure *A*.
			1—7 / 24	*B*	5	*Nil*	
			3—6 / 8—24	*C*	7	*Nil*	
iii	16th-17th Oct. 1936	9	2—5 / 7—8	*X*	4	2, *X*	
iv	28th Oct.-6th Nov. 1936	6—7	7—9 / 24	*A*	5	2, *C*; 1, *X*	The source was not pure *A*.
			3—13 / 5—24	*B*	16	5, *B*; 1, *AX*	
			4—12 / 5—24	*C*	24	3, *C*; 1, *X*; 2, *C*+*D*	
			4—7 / 24	*CX*	2	1, *CX*; 1, *CD*	
			26—33 / 21	*A*+*B*+*C*+*D*	4	2, *X*	The vectors were fed alternately on diseased plants of types *A*, *B*, *C* and *D* before being put on healthy plants.

Series	Time of experiment	Age of tobacco infected. (weeks)	No. of white-flies and period for which they were fed on diseased plant	Type of disease used as source	No. of plants infected	No. of plants which developed the disease of various types	Remarks
1	2	3	4	5	6	7	8
			No. / hrs.				
v	14th-16th Dec. 1936	8	3—16 / 6—24	*A*	8	*Nil*	
			3—11 / 6—24	*B*	8	2, *B*	
			5—13 / 6—24	*C*	8	2, *C*	
			4—14 / 6—7	*D*	8	*Nil*	
vi	25th Dec. 1936 to 5th Jan. 1937.	6—8	4—7 / 18	*A*	10	2, *A*?	
			2—5 / 24	*B*	8	1, *B*?	
			3—9 / 18—24	*C*	18	1, *C*	
			4—9 / 6	*D*	10	1, *D*?	
			2—8 / 24	*X*	8	1, *X*	
			11—24 / 6	*A*+*B*+*C*+*D*	7	3, *X*	The synthetic source as in Ser. *iv*.
			9—15 / 6	*A*+*C*	3	1, *X*	The synthetic source as in Ser. *iv*.
			7—11 / 6	*A*+*D*	3	2, *X*	The synthetic source as in Ser. *iv*.
vii	6th-9th Feb. 1937	7	4—11 / 18	*A*	15	1, *X*	
			4—12 / 18	*B*	15	4, *B*	
			5—13 / 18	*C*	15	*Nil*	
			6—11 / 18	*D*	20	3, *X*, 2, *D*?	
			5—9 / 24	*X*	15	5 *X*	

From the statement given above it will be noticed that though on the whole the percentage of positive results obtained was rather low, white-fly could transmit all types of leaf-curl from diseased to healthy tobacco individually and synthetically. Furthermore, it is evident that the fly was most capable of transmitting the disease between the end of September and the middle of November and that tobacco was most susceptible to developing the disease if it was infected when about eight weeks old. Both the time of the year and the stage of growth of tobacco plants when the infections are made thereto have important influence on the development of the disease. The percentages of positive results from infections made during second half of September, October-November and December-January, were 12, 35-50 and 12-18·7, respectively, the tobacco plants under experiment being about seven to nine weeks old. In the case of infections under series (II), which were made between 8-15th October, but with twelve to thirteen weeks old tobacco, the percentage of positive results was 6 only.

Tobacco plants in which leaf-curl of various types was developed by experiment are illustrated in colour in Plates IX-X, showing their characteristic features in detail. Figs. 3 and 4 are of the *B* and *C* types, and Fig. 2 of the leaves of *A* type; the *A* type plant was similar to that in Fig. 1 of which the source was sann-hemp. Figs. 8 and 7 are of *X* (synthetically produced from $A+B+C+D$) and of true *X* (as occurs in nature) respectively. The differences between the appearance of the two plants are noteworthy, indicating that the composition of *X* type considered to be mixture of all types by Pal and Tandon needs accurate determination.

In 1937 three large series of transmission experiments were performed to verify the results obtained in the previous year. In the 1937 season, however, tobacco plants available for experiments were rather poor in growth and did not react to infection properly. It is widely recognised that for proper reaction to virus infection the infected plant must be growing actively [Smith, 1935]. Moreover, as plants in the field did not show leaf-curl up to the middle of October no infections could be made before that date. Particulars of the various series are given in Table IV-B.

In the first series, seventeen infections using *D* type as source were done on nine weeks old seedlings. Four to nine specimens of white-fly which had been allowed to feed on the diseased source for seven to twenty-four hours, were put on each healthy plant. In thirty-two to thirty-four days two out of seventeen infected plants developed the disease of *X* type. In the same series three plants were infected at the same time as the above, but with *X* type as source. Four to six white-flies were allowed to feed on diseased tobacco for twenty to twenty-four hours. None of the infected plants developed leaf-curl.

In the second series thirty-five infections were done in the beginning (5-9th) of November, using different types of disease as source. Only two infected (with *C* type) plants developed disease in twenty-five and thirty

days. One was almost true to the source but recovered later and the second one showed symptoms of *X* type.

The third series was performed in the end of November (29th November to 1st December). Twenty-five infections were done, but none of the infected plants developed leaf-curl.

Thus white-fly was capable of transmitting leaf-curl from diseased to healthy tobacco plants though the percentage of positive results obtained was extremely low. This, as pointed out above, was perhaps due to the poor growth of the plants under experiment as well as to the fact that the infections were done rather late in the season.

VII. *Ageratum conyzoides*, a new important host of the tobacco leaf-curl virus

In December 1936 while making survey of the weeds which showed leaf-curl it was observed that *Ageratum conyzoides*, a very common weed at Pusa, was suffering from leaf-curl with symptoms resembling those of some types of diseased tobacco. Healthy leaf and diseased plant of the weed are illustrated in detail in Plate VII, figs. 5 and 5*a-b*. Some transmission experiments were performed on the 16-17th December to ascertain if this plant like sann-hemp was an alternate host of the tobacco leaf-curl virus and if the white-fly could transmit the disease from *Ageratum* to tobacco. The detailed particulars of the experiments are given in Table V. Six to sixteen specimens of white-fly were fed on diseased *Ageratum* for twenty-five hours and then transferred to a healthy tobacco seedling about nine weeks old. Six plants were infected in this way. About the 18th January two infected plants showed leaf-curl of *D* type. The other four seedlings and the controls were kept under observation up to the end of the season but did not show any symptoms of leaf-curl.

In 1937 four large series of experiments were performed to verify the above results. The particulars of these experiments are also detailed in Table V, and the results are briefly summarized below :—

In the first series, forty infections were made in the first week (5-8th) of September on about eight weeks old tobacco plants. Two to sixteen white-flies were fed on diseased *Ageratum* for sixteen to twenty-five hours before they were transferred to a healthy tobacco seedling. In the course of thirteen to eighty-three days, twenty-four out of forty infected plants developed leaf-curl, and in almost all the cases the disease was of *D* type.

About the end of September (26-28th), thirty more infections (second series) were made on about six weeks old seedlings. Six to twenty-six white-flies were used per plant and they were allowed to feed on diseased *Ageratum* for twenty to twenty-five hours. In eleven to thirty-three days, eight of the infected plants developed leaf-curl, in almost all cases the type again being *D*.

The third series consisted of twenty experiments in which five to thirteen white-flies which had fed on diseased *Ageratum* for twenty-four to forty-four hours were used per plant. The infections were done in the first week of November. Between twenty-four and thirty-three days, four of the infected plants developed leaf-curl of *D* type.

The fourth and the last series of experiments consisting of thirty infections was performed on six weeks old tobacco seedlings. One to eight specimens of white-fly were used per plant. They were kept on diseased *Ageratum* for twenty-four to forty-two hours. In the course of nineteen to thirty-two days, twelve of the infected plants developed leaf-curl of typical *D* type.

From the foregoing it will be evident that *Ageratum conyzoides* is an important alternate host of the tobacco leaf-curl virus *D* and that white-fly can transmit the disease from this host to tobacco very easily. In some of the experiments described above the percentage of positive result was as high as 60. It may be recalled that Thung [1934] working in Java also successfully transmitted leaf-curl virus from this weed to tobacco with a white-fly as vector, though the highest percentage of positive infections obtained by him was about 18 only. Massop [1932] also suspected a species of *Ageratum* to be a host of tobacco leaf-curl virus in Rhodesia.

VIII. Transmission of the disease from tobacco to *Ageratum conyzoides*

Having successfully transmitted the disease from *Ageratum* to tobacco with the help of white-fly, it was natural to ascertain if the disease could be transmitted back from tobacco to *Ageratum*. Therefore about the end of October (29-30th), 1937, a series of experiments was performed for this purpose. The particulars of the experiments are detailed in Table VI. The weed which had been specially grown for experimental purposes was about eleven weeks old at the time of infection. Two to nine specimens of white-fly were allowed to feed on diseased tobacco of *D* type and then transferred to a healthy seedling of the weed. Nine infections were made in this way. In fifteen to forty-seven days all the nine infected plants developed leaf-curl resembling that which occurs in the field (Plate VIII, fig. 4).

IX. Transmission of the disease from *Ageratum conyzoides* to sann-hemp and back to the weed

Sann-hemp and *Ageratum* having definitely proved to be alternate hosts of tobacco leaf-curl, infections were made to determine the relationship of the viruses in the two alternate hosts. In the first week of September 1937, eighteen sann-hemp plants about nine weeks old were infected. Two to seven white-flies which had been allowed to feed for eighteen to twenty-three hours on diseased *Ageratum* were put on each sann-hemp plant. In seventy-two to seventy-eight days two infected plants developed leaf-curl of S.V.2 type (Table VII). In one case side shoots also showed the disease.

On 11th and 12th October two series of experiments were performed to determine if the disease could be transmitted by white-fly from sann-hemp (of both S.V.1 and S.V.2 type) to *Ageratum*. Twenty out of twenty-one infected plants of the weed developed typical leaf-curl in 38-100 days (Table VIII). In Plate VIII, figs. 2 *a-b*, photographs of some of the plants in which the disease was produced by experiment are reproduced.

X. Transmission of the virus from diseased weed (*Ageratum conyzoides*) to healthy weed

Experiments were also performed to ascertain the role of white-fly as a transmitter of the virus from diseased to healthy weed. Twenty-four infections were made during the month of October 1937, using five to eleven specimens of the white-fly per plant, which had been allowed to feed on diseased weed for about twenty-four hours. In thirteen to seventy-one days, twenty-three out of twenty-four infected plants developed leaf-curl similar to that which occurs in nature. The particulars of the experiments are given in detail in Table IX. In Plate VIII, figs. 3 *a-b*, photographs of some plants in which the disease was produced by experiment and of two control plants (figs. 3 *c-d*) are reproduced.

Thus the experiments described above clearly show that *Ageratum conyzoides* is a very important alternate host of the tobacco leaf-curl virus and that white-fly can easily transmit the virus from this weed to tobacco and sann-hemp and back from these hosts to the weed as well as from diseased to healthy weed plants.

XI. Number of white-flies and their period of feeding on diseased source necessary for the transmission of leaf-curl and the incubation period of the disease inside the plant

We have not yet performed exact experiments to ascertain the minimum number of white-flies and the period of their feeding on diseased source which are necessary for successfully transmitting leaf-curl to tobacco, sann-hemp and *Ageratum*. Similarly, no special experiments have been performed to determine the minimum and maximum incubation periods of the disease inside the infected plants at different times of the year. However, the detailed particulars of the numerous experiments which are given in Tables I-IX, and which have been summarised in the previous pages, throw some light on the factors named above. The particular experiments under various series which give information about these factors are incorporated in the statements given below. The minimum number of white-flies tried and found successful in transmitting the disease to various hosts is shown in column 5 of the statements. The minimum period of time tried for which the known number (given in colunn 4) of white-flies fed on diseased source and transmitted the disease is shown in column 7, the minimum and maximum incubation periods of the disease inside the various plants are stated in columns 9 and 10. It was noticed that when the number of white-flies under trial

was the minimum, the period for which they fed to become viruliferous was not necessarily the least, neither was so the incubation period. Therefore for each pair of plants (the source and the infected plant) generally four experiments give the necessary information about the four factors named above, *viz.*, minimum number of white-flies tried, minimum period of feeding tried, minimum and maximum incubation periods of the disease. In a few cases only one experiment was found to give information about more than one factor. In such cases two or three experiments give the necessary information and have only been included in the statements. In the case of crops like tobacco and sann-hemp which have more than one type of leaf-curl, information on the factors referred to above is given separately for each type. The data given in the statements are briefly summarized below :—

Transmission of the disease from sann-hemp to tobacco (Statement I)

The least number of individuals tried which successfully transmitted the disease of S.V.2 type was five. They had been kept on the diseased source for twenty-five hours and the incubation period of the disease in this case was thirty-six days which proved to be the maximum for this type. The minimum period of feeding on diseased source in the case of successful transmission was $20\frac{1}{2}$ hours, the number of specimens used being ten. A smaller number was not tried. The minimum incubation period was eleven days and was so in the case of the experiment in which the white-flies had fed for the minimum period.

With diseased sann-hemp of S.V.1 type as source, the least number of specimens tried which successfully transmitted the disease was six. The minimum period of feeding was $18\frac{1}{2}$ hours, using seventeen individuals. The maximum and minimum incubation periods were eighty-four and eight days respectively. It is noteworthy that in the case of both types the minimum incubation period of the disease inside tobacco was noted when the plants were infected towards the end of September (27-29th September, 1937).

Transmission of leaf-curl from tobacco to sann-hemp (Statement I)

Type A.—The minimum number of white-flies tried and found successful was two. They had fed for about seventeen hours. The minimum feeding period successfully tried was thirteen hours, using three specimens. The incubation period varied between 53 and 112 days, the time of infection being from the end of July up to end of August.

Statement I

Serial No.	Time and source of infection and infected plant	Type of disease of source	No. of white-flies used	Minimum No. of white-flies tried which transmitted disease	Feeding period	Minimum feeding period tried resulting in successful transmission	Incubation period	Minimum incubation period	Maximum incubation period	Type of disease appearing and remarks
1	2	3	4	5	6	7	8	9	10	11
					Hrs.		Days	Days	Days	
	Sann-hemp to tobacco									
1	24th September 1936 .	S.V.2 .	5	5	25	...	36	...	36	*A*
2	27th September 1937 .	Do. .	10	...	20½	20½	11	11	...	*A*
3	27th September to 1st November 1937.	Do. .	7—13	...	20½—24	...	11	11	...	*A*
1	14th December 1936 and 9th September 1937.	S.V.1 .	6	6	24, 29	...	39, 63, 13	...	...	*X*
2	4th November 1936 .	Do. .	26	...	...	18½	84	...	84	*X*
3	29th September 1937 .	Do. .	13—27	...	48¾	...	8	8	...	*X*
	Tobacco to sann-hemp									
1	27th to 30th August 1937	*A* . .	2	2	16½—17	...	53, 84	53	...	S.V.2
2	21st July 1937 . .	*A* . .	3	...	13	13	...	...	112	
1	16th July 1937 .	*X* . .	6	...	6	6	117	117	...	
2	18th July 1937 . .	*X* . .	3	3	8	...	122	...	122	
	Sann-hemp to sann-hemp									
1	3rd September 1937 .	S.V.1 .	2	2	20	...	71	...	...	
2	5th November 1936 .	Do. .	24	...	19	19	31	...	...	
3	11th October 1936 .	Do. .	10	...	24	...	27	27	...	
4	13th October 1936 .	Do. .	5	...	24	...	136	...	136	

Type X.—The minimum number of white-flies tried which successfully transmitted the disease was three. They had fed for eight hours and the incubation period in that case was 122, which was the maximum for this type. The minimum feeding period tried was six hours, the number of individuals used being three.

Transmission of the disease from sann-hemp to sann-hemp (Statement I)

The minimum number of specimens tried and found successful was two. They had fed on diseased source for twenty hours. The minimum period for which white-flies were fed and transmitted disease was nineteen hours. A shorter feeding period was not tried. The incubation period varied between 27 and 136 days.

Transmission of the disease from tobacco to tobacco (Statement II)

Type A.—The least number of individuals tried and found successful was three. They had fed for eight hours. which was the minimum period tried for this type. The incubation period varied between seventeen and ninety-four days, the minimum and maximum being with the infections done in the beginning of February and during December respectively.

Type B.—The minimum number of white-flies tried and found successful was six. They had fed for twenty-four hours. The minimum feeding period tried was six hours, using six to seven individuals. The incubation period varied between 18 and 110 days, the minimum again being in the case of the infection which was done in the beginning of February.

Type C.—The minimum number of specimens and the minimum period of feeding tried and found successful were five, and five hours respectively. The incubation period varied between twelve and fifty-nine days, the minimum and maximum being in the case of the infections done in the end of October and during December respectively.

Type D.—The least number of specimens tried which transmitted the disease was four. They had fed for six hours, which was the minimum period tried for this type. The incubation period varied between twenty and ninety-four days, the minimum and maximum being in the case of the infections which were done in the beginning of February and during December respectively.

When the disease was produced synthetically by feeding the white-fly on types *A*, *B*, *C* and *D* successively, the minimum number of specimens which were tried and transmitted the disease was thirteen, the minimum feeding period tried being six hours on each type. The incubation period varied between 37 and 140 days.

From the foregoing it will be clear that as few as two flies could successfully transmit the disease. The minimum feeding period tried giving rise to successful transmission was five hours. The incubation period varied from 12 to 140 days, the minimum and maximum being generally in the case of those infections which were done in the beginning of February and during December respectively.

Statement II

Serial No.	Time and source of infection and infected plant	Type of disease of source	No. of white-flies, used	Minimum No. of white-flies tried which transmitted disease	Feeding period	Minimum feeding period tried resulting in successful transmission	Incubation period	Minimum incubation period	Maximum incubation period	Type of disease appearing and remarks
1	2	3	4	5	6	7	8	9	10	11
					Hrs.		Days	Days	Days	
	Tobacco to tobacco									
1	15th October 1936 .	*A* . .	3	3	8	8	26	...	...	*A* ? Source also slightly mixed.
2	6th February 1937 .	*A* . .	9	...	18	...	17	17	...	*A* ?
3	25th December 1936 . .	*A* . .	5	...	18	...	94	...	94	*X*
1	1st November 1936 .	*B* . .	6	6	24	...	36	...	...	*B* ?
2	16th December 1936 and 4th January 1937.	*B* . .	6—7	...	6	6	68	...	...	*B*
3	6th February 1937 .	*B* . .	7	...	18	...	18	18	...	*B*
4	1st November 1936 .	*B* . .	11	...	24	...	110	...	110	*B*
1	31st October 1936 to 16th December 1936.	*C* . .	5	5	24—26	...	37—59	...	59	*C*
2	28th October 1936 .	*C* . .	10	...	5	5	18	...	...	*C*
3	31st October 1936 .	*C* . .	7	...	24	...	12	12	...	
1	26th December 1936 and 14th October 1937.	*D* . .	5	4	6—7	6	94, 34	...	94	*D* ?
2	6th February 1937 .	*D* . .	7	...	18	...	20	20	...	*D*
1	16th October 1936 .	*X* . .	2	2	7½	7½	25	...	25	*X*
2	8th-9th February 1937.	*X* . .	5	...	24	...	11	11	...	*X*
1	27th December 1936 .	*A*+*B*+*C*+*D*	13	13	6	6	52	...	...	*X*
2	Ditto . .	Do. .	21	...	6	...	37	37	...	*X*
3	5th November 1936 .	Do. .	26	...	21	...	140	140	140	*X*

Statement III

Serial No.	Time and source of infection and infected plant	Type of disease of source	No. of white-flies used	Minimum No. of white-flies tried which transmitted disease	Feeding period	Minimum feeding period tried resulting in successful transmission	Incubation period	Minimum incubation period	Maximum incubation period	Type of disease appearing and remarks
1	2	3	4	5	6	7	8	9	10	11
					Hrs.		Days	Days	Days	
	Ageratum to tobacco									
1	22nd November 1937	...	1	1	24	...	19	...	...	*D*
2	7th -8th September 1937	...	5—16	...	16	16	13—83	...	...	*D*
3	Ditto	...	8	...	16	...	13	13	...	*D*
4	Ditto	...	11	...	16	...	83	...	83	*D*
	Tobacco to Ageratum									
1	29th and 30th October 1937	*D*	2—9	2	16	16	15—47	...	...	Resemble that in the field.
2	Ditto	...	...	...	...	...	15	15	...	
3	Ditto	...	...	...	...	...	47	...	47	
	Ageratum to sann-hemp									
1	3rd September 1937	...	3	3	23	...	78	...	78	S. V. 2
2	4th September 1937	...	5	...	18	18	72	72	...	S. V. 2.
	Sann-hemp to Ageratum									
1	11th-12th October 1937	S. V. 1	5—12	5	24	24	49—69	...	...	Type resembles that in the field.
2	Ditto	Do.	...	..	...	...	49	49	...	
3	Ditto	Do.	...	...	...	...	69	...	69	
1	Ditto	S. V. 2	5—11	5	24	24	38—100	...	...	
2	Ditto	Do.	...	...	24	...	38	38	...	
3	Ditto	Do.	...	...	24	...	100	...	100	
	Ageratum to Ageratum	...	5—11	5	24	...	13—71	...	...	
1	10th and 11th October 1937	...	...	...	...	...	13	13	...	
2	Ditto	...	...	...	...	...	71	...	71	
3	29th-31st October 1937.	...	6—10	...	22—24	22	27—39	...	...	

Transmission of the disease from Ageratum *to tobacco, sann-hemp,* Ageratum *etc.* (*Statement III*)

Even one white-fly which had been fed for twenty-four hours on diseased *Ageratum* successfully transmitted the disease to tobacco. However, the minimum feeding period successfully tried was sixteen hours, using five individuals. The incubation period varied between thirteen and eighty-three days.

In the case of tobacco to *Ageratum*, the minimum number of individuals successfully tried was two. They had fed for sixteen hours.

The minimum incubation period with tobacco and *Ageratum* as host and source respectively and *vice versa* was generally thirteen to fifteen days. In the case of *Ageratum* and sann-hemp, however, it was thirty-eight to forty-nine days.

XII. Other food plants of white-fly, *Bemisia gossypiperda* M. & L.

In addition to tobacco, *Ageratum conyzoides* and sann-hemp mentioned in the previous pages, white-fly has been found feeding and breeding on a variety of plants, both cultivated and wild, in the neighbourhood of Pusa. The list of various plant hosts is given below, and those which have been observed to suffer from some kind of leaf-curl have been marked with an asterisk :—

Cultivated plants

* *Althaea rosea* (hollyhock)
Cannabis sativa (*ganja*)
Hibiscus cannabinus
H. esculentus (lady's fingers)
Gossypium herbuceum (cotton)
* *Cucumis sativa* (cucumber)
C. melo (melon)
* *Lagenaria vulgaris* (bottle gourd)
Trichosanthes dioica
**Lycopersicum esculentum*
Nicotiana glauca
N. rustica
N. glutinosa
Capsicum annuum
Solanum tuberosum (potato)
S. melogena (brinjal)
Glycine hispida (soya bean)
* *Phaseolus calcaratus*
P. radiatus (green gram)
P. mungo (black gram)
Medicago sativa (lucerne)
Trifolium alexandrinum (berseem)
* *Zinnia elegans*
* *Petunia* sp.

Wild plants

Hibiscus rosasinensis (shoe flower)
* *Sida rhombifolia*
Nicotiana plumbaginifolia (wild tobacco)
**Solanum nigrum*
S. verbasiofolium
* *Datura stramonium* (datura)
Launea asplenifolia
Duranta plumieri (duranta)
Euphorbia hirsuta
Anisomeles ovata (wild bur)
Nepeta ruderalis
* *Scoparia dulcis*
Vernonia cinerea var. anthelmentica
Ocimum sanctum
Ocimum basilicum.

Cultivated plants

Arachis hypogea (groundnut)
Cajanus cajan (red gram)
Luffa acutangula (ribbed gourd)
Cucurbita maxima (pumpkin)

As is well known this white-fly is a serious pest of cotton in the Punjab. Husain and Trehan [1936] have given a complete list of its host plants in that province. However, this white-fly is not reported to cause leaf-curl in cotton or tobacco in the Punjab. Whereas cotton has been carefully examined for leaf-curl, a survey of tobacco areas for the incidence of leaf-curl is warranted, as one of us (H. S. P.) has recently observed a crop of tobacco badly affected by leaf-curl of all types near Agra in the western United Provinces.

XIII. Discussion and conclusions

From the observations described in the previous pages the following conclusions can be drawn :—

Sann-hemp is an important alternate host of the tobacco leaf-curl viruses *A* and *X*, the two viruses causing different symptoms in sann-hemp, giving rise to two types of disease, which have been designated as S. V. 1 and S. V. 2 in the paper. White-fly, *Bemisia gossypiperda*, can easily transmit both types of disease from sann-hemp to tobacco.

Though white-fly can transmit leaf-curl from tobacco back to sann hemp, it does not however seem to be a very efficient vector in this respect Moreover, in view of the fact that the times of growth of the two crops are different, tobacco does not seem to serve as a source of infection for the disease in sann-hemp.

The weed *Ageratum conyzoides* is another important alternate host of tobacco leaf-curl, especially of *D* type. White-fly is capable of easily transmitting the disease from this host to tobacco. White-fly can also easily transmit the disease from tobacco back to the weed. Sometimes 100 per cent positive transmissions were obtained. White-fly is capable also of easily transmitting the disease from sann-hemp to *A. conyzoides*, though it cannot transmit the disease back to sann-hemp so easily.

Regarding the role of white-fly as a vector of leaf-curl from diseased to healthy tobacco, the results of two years' experiments show that white-fly could transmit the disease only in a few cases. Therefore, it appears that in nature diseased tobacco is not an important source of infection for healthy tobacco, and that the alternate hosts referred to above, *viz.*, sann-hemp and *A. conyzoides* seem to play a much more important part in this connection. These two hosts harbour leaf-curl viruses *A*, *D* and *X* types ; the alternate hosts, if any, for *B* and *C* types remain yet to be discovered. In Java also it has been shown [Thung, 1932 ; 1934] that the chief source of infection to tobacco lies outside the crop.

It appears that tobacco is most susceptible to getting the infection if it is about eight weeks old and if the infection takes place in autumn or in February. Sann-hemp is most susceptible during July and August, when

it is about two to three months old. Wille [1928] working on ' leaf-crinkle ' of sugar beet also concluded that the disease is very much influenced by the age of the host plant, while Spencer [1938] has recently observed seasonal variations in susceptibility of tobacco to infection with tobacco-mosaic virus. Regarding the autumn and spring seasons being more suitable than winter for the infection of tobacco *, it is likely that the air temperature and humidity prevailing during these seasons are conducive to the development of the disease. In this respect the case of tobacco leaf-curl seems to be similar to that of mosaic disease of some Cruciferae in the United States, which is very severe if the temperature is 70°-80° F., whereas at 55°-65°F. even more susceptible hosts cease to show mosaic symptoms. It may also be recalled that ' yellow dwarf ' of potatoes in America develops severe symptoms at 25°C., while no symptoms appear at 15°C. [Goss and Peltier, 1925]. The ' curly top ' of sugar beet also behaves similarly [Smith, 1933]. Grainger [1936] has recently observed the same phenomenon in the case of tobacco mosaic. However, there are numerous cases on record [Smith, 1933] in which opposite is the case, *viz.*, low temperatures accentuate the symptoms of the disease, whereas higher temperatures suppress them.

The incubation period of the disease inside the infected plant seems to be connected with the question of the accentuation or suppression of the symptoms of the disease discussed above. It was noticed that with the age of infected tobacco remaining the same, the incubation period was generally minimum if the infections were done during September and October or in February when the air temperature starts rising at Pusa. The incubation period was generally the maximum if the infections were done during December. Spencer [1938] has recently found the same in the case of tobacco-mosaic virus. Apart from air temperature affecting the plant, it is quite likely that white-fly is most viruliferous at these times of the year, which again may be due to several environmental factors including temperature and humidity. Storey [1933] attached great importance to the influence of weather condition on the ability of the insect vector to take up and transmit viruses.

We have not performed exact experiments to determine the length of time necessary for white-fly to feed on the diseased host for successfully transmitting the disease to healthy host, but the general transmission experiments show that white-fly can transmit the virus after five to six hours' feeding, shorter periods not having been tested. Kirkpatrick [1931] found this period to be about 4½ hours in the case of the white-fly (*Bemisia* sp.) which spreads leaf-curl of cotton in Sudan. Storey [1928] showed that the leaf-hopper *Cicadulina mbila* could acquire the maize streak virus after one hour's feeding, whereas Doolittle and Walker [1928] concluded that *Aphis gossypii* could pick up the cucumber mosaic virus after feeding for five minutes only. Recently, Watson [1936] has obtained the highest proportion of infection after two to five minutes feeding by *Myzus persicae* on the infected plants.

* We could not perform any experiment during the hottest season of the year (April-June) as tobacco crop is not grown at that time in Bihar.

The minimum number of white-flies tested and found successful for the transmission of the disease from sann-hemp to tobacco was five; for the transmission from tobacco to sann-hemp and from sann-hemp to sann-hemp the number was two. Fewer numbers in all these cases were not tested. In the case of *Ageratum* to tobacco one white-fly was found to be able to transmit the disease.

It was observed that with the minimum number of white-flies tried which successfully transmitted leaf-curl, the incubation period of the disease inside the infected plant was generally not the shortest. It appears that the period of feeding remaining the same, there is no relation between the number of infected individuals used and the incubation period of the disease.

In addition to sann-hemp and *Ageratum conyzoides*, we have observed white-fly breeding on a number of plants, some of which (Cucurbits, hollyhock, zinnia, *Scoparia*, *Sida* sp., etc.) show leaf-curl, which may be closely allied to, if not identical with, tobacco leaf-curl. Therefore it appears that unlike most viruses, the tobacco leaf-curl viruses may have a big host range and thus resemble those of aster yellows, tomato spotted wilt, tobacco ring spot, etc. If this is true, the problem of the control of the disease by the eradication of alternate host plants becomes very difficult and the chances of success by keeping white-fly under check with insecticides appear to be greater.

XIV. Acknowledgments

We are thankful to the Imperial Economic Botanist for making arrangements for the supply of tobacco seedlings for our experiments and for affording other facilities at the Botanical Substation at Pusa. Mr. K. L. Khanna, Sugarcane Expert, Bihar, Pusa, was kind enough to loan us the services of his photographer for a few days.

REFERENCES

Doolittle, S. P. and Walker, M. N. (1928). Abstr. in *Phytophatt.* **18**, 143
Goss, R. W. and Peltier, G. L. (1925). *Nebroska Agric. Expt. Stn. Res. Bull.* No. **29**
Grainger, J. (1936). *Nature* **137** (3453), 31-32
Husain, M. A.; Trehan, K. N. and Verma, P. M. (1936). *Ind. J. Agric. Sci.* **6**, 893-903
Kirkpatrick, T. W. (1931). *Bull. Ent. Res.* **22**, 323-363
Massop, (1932). *Rhod. Agric. J.*, **29**, 869-872
Pal, B. P. and Tandon, R. K. (1937). *Ind. J. Agric. Sci.* **7**, 363-393
Pruthi, H. S. and Samuel, C. K. (1937). *Ind. J. Agric. Sci.* **7**, 659-670
Smith, K. M. (1931). *Biol. Rev.* **6**, 302-44
———— (1933). "Recent Advances in the Study of Plant Viruses." Churchill, London
———— (1935). "Plant Viruses". Methuen, London
Spencer, E. L. (1938). *Phytopl.* **28**, 147-150
Storey, H. H. (1928). *Ann. Appl. Biol.* **15**, 1-25
———— (1933). *Proc. Roy. Soc.* **B, 113**, 463-485
———— (1935). *East Afr. Agric. J.* **1**, 148-53
Thung, T. H. (1932). *Meded. Proefst. Vorsteulandsche Tobak, No.* **72**, 54 pp.
———— (1934). *Meded Proefst. Vorsteulandsche No.* **78**, 18 pp.
Watson (Hamilton), M.A. (1936). *Phil. Tran. Roy. Soc. Lond.* **B, 226**, 540, 452-489
West, J. (1936). *Trop. Agric. (Trinidad)*, **13**, 242-44
Willie, J. (1928) *Arb. biol. Reichsanst. L and—u. Forstw.* **16**, 1, 115-167

PLATE VII

FIG. 1. Sann-hemp plant suffering from leaf-curl of S. V. 1 type

FIG. 2. Sann-hemp plant suffering from leaf-curl of S. V. 2 type

FIG. 2-*a* Leaf of the same, magnified

FIG. 3. A leaf of a typical sann-hemp plant, free from leaf-curl, magnified

FIG. 4. Sann-hemp plant in which leaf-curl was produced by experiment with diseased tobacco (*A* type) as source of infection

FIG. 4-*a* Leaf of the same, magnified

FIG. 5. A twig of *Ageratum conyzoides* suffering from leaf-curl

FIG. 5-*a* A leaf of the same, enlarged

FIG. 5-*b* A leaf from healthy twig

5b
5
5a
4
4a
2
2a
3
1

PLATE VIII

1. A series of tobacco plants (a—h) in which leaf-curl was produced by experiment with diseased sann-hemp as source of infection, j & k two typical control plants

2. Photos of some *Ageratum* plants (a—b) in which leaf-curl was produced by experiment, with diseased sann-hemp as source of infection

3. Photos of some *Ageratum* plants (a—b) in which leaf-curl was produced by experiment, with diseased *Ageratum* as source of infection ; plants c and d are controls of these as well as of the plants in Fig. 2 above

4. Photo of an *Ageratum* plant in which leaf-curl was produced by experiment, with diseased tobacco as source of infection.

1
a
b
c
d
e
f
g
h
j
k
2
a
b
3
a
b
c
d
4

1
1a
2
2a
3
4
5

PLATE IX

1. A tobacco plant in which leaf-curl of *A* type was produced by experiment with diseased sann-hemp as source of infection

1-*a*. Lower side of a leaf of the same—note the cup-shaped enations

2 & 2*a*. Lower and upper sides of the leaves of a tobacco plant in which leaf-curl of *A* type was produced using similarly diseased tobacco plant as source of infection

3. A tobacco plant in which leaf-curl of *B* type was produced by experiment with similarly diseased tobacco plant as source of infection

4. A tobacco plant in which leaf-curl of *C* type was produced by experiment with similarly diseased tobacco plant as source of infection

5. The white-fly, *Bemisia gossypiperda*, laying eggs on a tobacco leaf

PLATE X

6. A tobacco plant in which leaf-curl of *D* type was produced by experiment with similarly diseased tobacco plant as source of infection

6-*a*. A leaf of the same, magnified

7. A tobacco plant in which leaf-curl of *X* type was produced, with a similarly affected tobacco plant as source of infection

8. A tobacco plant in which leaf-curl was produced by experiment with tobacco plants suffering from *A*, *B*, *C* and *D* types as source of infection

8-*a*. Lower surface of a leaf of the plant in FIG. 3, magnified (Note the prominent enations)

6a
6
7
8a
8

TABLE I-A—*contd.*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series iv							
Time of experiment, 3rd-4th November 1936.	1—7	...	3rd Nov. 1936—11 A.M.	24	7—17	...	Negative.
Age of diseased sann-hemp (Source) : 17 weeks.	...	...	...	...	...	...	
Age of healthy tobacco infected : 16 weeks.							
Series v							
Time of experiment, 14th December 1936	1	...	14th Dec. 1936—10 A.M.	24	6	39	Positive ; type *X*.
Age of diseased sann-hemp (Source) : 22 weeks.	2	...	Do. . .	24	6	63	Positive, A type of *X*.
Age of healthy tobacco infected : about 8 weeks.	3—10	...	Do. . .	24	3—7	...	Negative.

None of the controls of the various series developed leaf-curl disease

TABLE I-B

Particulars of experiments performed to transmit the disease from sann-hemp to tobacco during 1937

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment, 9th-11th Sept. 1937	1	S. V. I .	9th Sept. 1937—10-30 A.M.	29	14	51	Positive ; type *X*
Age of diseased sann-hemp (Source) : 13 weeks.	2	Do. . .	Do. . .	29	8	22	Positive ; type *D* ?
	3	Do. . .	Do. . .	29	7	62	Positive ; type *X*
Age of healthy tobacco infected : 8 weeks.	4	Do. .	Do. . .	29	10	28	Do.
	5	Do. . .	Do. . .	29	7	40	Do.
	6	Do. . .	Do. . .	29	8	40	Do.
	7	Do. . .	Do. . .	29	7	18	Do.
	8	Do. . .	Do. . .	29	10	18	Do.
	9	Do. . .	Do. . .	29	10	22	Positive ; type *D* ? (Recovered).
	10	Do. . .	Do. . .	29	7	22	Positive ; type *X*.
	11	Do. . .	Do. . .	29	11	18	Do.
	12	Do. . .	Do. . .	29	6	13	Do.
	13—27	Do. . .	10th Sept. 1937—4 P.M.	24	6—13	...	Negative.
	28	Do. . .	Do. . .	24	9	17	Positive ; type *X* ? (Recovered)
	29	Do. . .	Do. . .	24	11	17	Do.
	30—32	Do. . .	Do. . .	24	8—9	...	Negative.

TABLE I-B—*contd.*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i—contd.	33	S. V. I.	10th Sept. 1937—4 P.M.	24	7	17	Positive; type *X* (Recovered).
	34—39	Do. . .	Do. . .	24	6—14	...	Negative.
	40	Do. . .	Do. . .	24	10	32	Positive; type *X*.
Series ii							
Time of experiment, 27th September to 1st October 1937	1	S. V. 1 .	27th Sept. 1937—11 A.M.	20½	19	11	Positive; type *X*.
Age of diseased sann-hemp (Source): about 15 weeks.	2	Do . .	Do. . .	20½	21	15	Do.
	3	Do. . .	Do. .	20½	17	11	Do.
Age of healthy tobacco infected: about 7 weeks.	4	Do. . .	Do. . .	20½	11	...	Negative.
	5	Do. . .	Do. . .	20½	9	...	Do.
	6	Do. . .	Do. . .	20½	14	15	Positive; type *X*.
	7	Do. . .	Do. . .	20½	8	11	Do.
	8	Do. . .	Do. . .	20½	11	11	Do.
	9	Do. . .	29th Sept. 1937—10 A.M.	48¾	11	12	Do.
	10	Do. . .	Do. . .	48¾	25	8	Do.
	11	Do. . .	Do. . .	48¾	22	12	Do
	12	Do. . .	Do. . .	48¾	21	11	Do.
	13	Do. . .	Do. . .	48¾	16	...	Negative.
	14	Do. . .	Do. . .	48¾	17	11	Positive; type *X*.

	15	Do. . .	Do. . .	48¾	13	8	Do.
	16	Do. . .	Do. . .	48¾	12	...	Negative.
	17	Do. . .	Do. . .	48¾	23	11	Positive ; type *X*.
	18	Do. . .	Do. . .	48¾	22	11	Do.
	19	Do. . .	Do. . .	48¾	9	...	Negative.
	20	Do. . .	Do. . .	48¾	24	11	Positive ; type *X*.
	21	Do. . .	Do. . .	48¾	23	8	Do.
	22	Do. . .	Do. .	48¾	18	14	Do.
	23	Do. . .	Do. . .	58¾	14	11	Do
	24	Do. . .	Do. .	48¾	19	12	Do.
	25	Do. . .	Do. .	48¾	26	8	Do.
	26	Do. . .	Do. . .	48¾	27	8	Do.
	27	Do. . .	Do. . .	48¾	14	25	Do.
Series iii							
Time of experiment, 27th-29th September 1937.	1	S. V. 2 .	27th Sept. 1937—11 A.M.	20½	10	11	Positive ; type *A*.
Age of diseased sann-hemp (Source) : about 15 weeks.	2	Do. . .	Do. . .	20½	13	...	Negative.
Age of healthy tobacco infected : about 7 weeks.	3	Do. . .	29th Sept. 1937—10 A.M.	48¾	27	12	Positive ; type *A*.
Series iv							
Time of experiment, 31st Oct.-2nd November 1937.	1	S. V. 2 .	31st Oct. 1937—Noon.	29	25	19	Positive ; type *A*.
Age of diseased sann-hemp (Source) : 18 weeks.	2	Do. . .	Do. . .	29	5	...	Negative.
	3	Do. . .	Do. . .	29	9	13	Positive ; type *A*.
Age of healthy tobacco infected : 7 weeks.	4	Do. . .	Do. . .	29	8	19	Do.
	5	Do. . .	Do. . .	29	6	...	Negative.
	6	Do. . .	Do. . .	29	11	13	Positive ; type *A*.
	7	Do. . .	Do. . .	29	7	12	Do.

TABLE I-B—*concld.*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series iv—contd.	8	S. V. 2.	1st Nov. 1937—11 A.M.	24	6	...	Negative.
	9	Do. . .	Do. . .	24	16	...	Do.
	10	Do. . .	Do. . .	24	7	11	Positive ; type *A*.
	11—15	Do. .	Do. . .	24	5—16	...	Negative.
	16	Do. .	Do. .	24	10	16	Positive ; type *A*.
	17	Do. . .	Do. . .	24	7	21	Do.
	18	Do. . .	Do. . .	24	9	...	Negative.
	19—22	Do. . .	1st Nov. 1937 4 P.M.	24	11—13	11	Positive ; type *A*.
	23—25	Do. . .	Do. . .	24	7—14	14—15	Do.
	26	Do. . .	Do. . .	24	11	...	Negative.
	27	Do. . .	Do. . .	24	9	...	Do.
Series v							
Time of experiment, 25th-28th November 1937.	1	S. V. 2 .	25th Nov. 1937—11 A.M.	24	6	27	Positive ; type *A*.
Age of diseased sann-hemp (Source) : 23 weeks.	2—25	Do. . .	25th-28th Nov. 1937.	24	3—7	...	Negative.
Age of healthy tobacco infected : 6 weeks.							

None of the controls of the various series developed leaf-curl disease.

TABLE II-A

Particulars of experiments performed to transmit the disease from tobacco to sann-hemp during 1936

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment 12th-22nd September 1936	1—3	*A*	12th-14th Sept. 1936—4 P.M.	24—48	3—10	...	Negative.
Age of diseased tobacco (Source): 13—14 weeks. Age of sann-hemp inoculated: 9—10 weeks.	4—5	*C*	21st-22nd Sept. 1936—5 P.M.	24	3—5	...	Do.
Series ii							
Time of experiment: 24th Sept.—3rd Oct. 1936	1—7	*X*	24th Sept.-3rd Oct. 1936.	17—24	1—8	...	Do.
Age of diseased tobacco (Source): 13—14 weeks. Age of sann-hemp inoculated: 10—12 weeks.	8—11	*C*	29th-30th Nov. 1936—5 P.M.	24	8—12	...	Do.
Series iii.							
Time of experiment, 13th October 1936	1—3	*B*	13th Oct. 1936—9 A.M.	8	2—7	25	Slight reaction perceptible The lower three leaves of inoculated plant were curled and drooping.
Age of diseased tobacco (Source): about 13 weeks. Age of sann-hemp inoculated: 13 weeks.	4—6	*B*	Do.	8	2—7	...	Negative.
Series iv							
Time of experiment, 5th-6th November 1936	1—8	*C*	5th-6th Nov. 1936	8—22	7—31	...	Do.
Age of diseased tobacco (Source): 17 weeks. Age of sann-hemp inoculated: 16 weeks.	9—10	*A*	Do.	22	11—13	...	Do.

None of the controls in the various series developed leaf-curl disease.

TABLE II-B

Particulars of experiments performed to transmit the disease from tobacco to sann-hemp during 1937

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment, 16th-17th July 1937	1—4	*A*	16th-17th July 1937	16	2—4	...	Negative.
	5—9	*X*	Do. .	16	2—4	...	Do.
Age of diseased tobacco (of 1936-37 season as Source): 44 weeks.	10	*C*	Do. .	16	4	...	Do.
	11	*X* of *C*	Do. .	4	5	...	Do.
Age of healthy sann-hemp inoculated : 2½ weeks.	12	*C* & *D*	Do. .	4	5	...	Do.
	13	*X*	Do. .	6	6	117	Positive ; type S. V. I.
	14	*X*	Do. .	6	6	...	Negative.
	15—16	*C*	Do. .	6	6	...	Do.
	17	*A*	Do.	4	5	...	Do.
Series ii							
Time of experiment, 18th-23rd July 1937.	1	*C*	18th July 1937—8 A.M.	8	3	...	Do.
Age of diseased tobacco (1936-37 season as Source) : 36 weeks.	2	*C*	Do. .	8	3	119	Positive ; type S. V. I.
	3	*C*	Do. .	10	7	...	Negative.
Age of healthy sann-hemp inoculated : 2½—3 weeks.	4	*A*	Do. .	8	3	...	Do.
	5	*X*	Do. .	8	3	122	Positive ; type S. V. I.
	6	*X*	Do. .	10	7	...	Negative.

	7	X	Do.	10	7	118	Positive ; type S. V. I.
	8	X	Do.	10	7	...	Negative.
	9	A	21st July 1937—6 P.M.	13	3	...	Do.
	10	A	Do.	13	3	112	Positive ; type S. V. 2.
	11—20	A	Do.	13	3	...	Negative.
	21	C	23rd July 1937—9 A.M.	8	4	...	Do.
	22	C	Do.	8	4	...	Do.
Series iii.							
Time of experiment, 23rd-29th July 1937.	1—3	C	23rd July 1937—9 A.M.	8	4	...	Negative.
Age of diseased tobacco (1936-37 Season as Source) : 32—40 weeks.	4	C	Do.	8	4	133	Positive ; type S. V. I.
	5	C	Do.	8	4	...	Negative.
Age of healthy sann-hemp infected : 3—4 weeks.	6	C	Do.	8	4	131	Positive ; type S. V. I.
	7—12	A	24th-26th July 1937.	6—18	2—5	...	Negative.
	13—16	X	26th July 1937	6	4—5	...	Do.
	17—20	A	27th July 1937—11 A.M.	6	4—9	...	Do.
	21	D	29th July 1937—11 A.M.	7	4	...	Do.
	22	D	Do.	7	3	...	Do.
	23	D	Do.	7	3	...	Do.
	24	C	Do.	7	1	...	Do.
	25	C	Do.	7	3	117	Positive ; type S. V. I.

TABLE II-B—*contd.*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series iv							
Time of experiment, 27th-31st August 1937.	1—2	*C*	27th Aug. 1937—5 P.M.	17	3	...	Negative.
Age of diseased tobacco (1936-37 season as Source): 49—50 weeks.	3	*C*	Do.	17	4	144	Positive ; type S. V. I.
	4	*C & D*	Do.	17	2	68	Do.
Age of healthy sann-hemp infected : 8—8½ weeks.	5—6	*C & D*	Do.	17	2—4	...	Negative
	7	*A*	Do.	17	3	53	Positive ; type S. V. 2
	8	*A*	Do.	17	2	53	Do.
	9—10	*X*	Do.	17	2—4	...	Negative.
	11—13	*C & D*	28th Aug. 1937—11 A.M.	6	3—4	...	Do.
	14—16	*A*	Do.	6	3—5	...	Do.
	17	*A*	30th Aug. 1937—5 P.M.	16½	3	76	Positive ; type S. V. 2.
	18	*A*	Do.	16½	2	...	Negative.
	19	*A*	Do.	16½	4	70	Positive ; type S. V. 2.
	20	*A*	Do.	16½	2	84	Do.
	21—22	*A*	Do.	16½	3—4	...	Negative.
	23	*A*	Do.	16½	5	70	Positive ; type S. V. 2.
	24	*A*	Do.	16½	4	...	Negative.

None of the controls of the various series developed leaf-curl disease.

TABLE III-A

Particulars of experiments performed to transmit the disease from sann-hemp to sann-hemp during 1936

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment, 8th-13th October 1936	1	...	8th Oct. 1936—5-30 P.M.	24	4	57	Positive; almost similar to the source. Side shoots also were diseased.
Age of diseased sann-hemp (Source): 14—15 weeks.	2—6	...	Do.	24	5—9	...	Negative.
Age of healthy sann-hemp infected: 13—14 weeks.	7	...	11th Oct. 1936—2 P.M.	24	10	29	Positive; almost similar to the source. Side shoots also were diseased.
	8	...	Do.	24	10	27	Ditto.
	9—18	...	Do.	24	10—12	...	Negative.
	19	...	12th-13th Oct 1936—11 A.M.	24	5	136	Positive; almost similar to the source, side shoots also were diseased.
	20—24	...	Do.	24	5—7	...	Negative
Series ii							
Time of experiment, 4th/5th November 1936	1	...	4th-5th Nov. 1936—4 P.M.—11 A.M.	19	24	31	Positive; almost similar to the source, side shoots also were diseased.
Age of diseased sann-hemp (Source): 17 weeks.							
Age of healthy sann-hemp infected: 17 weeks.	2—10	...	Do.	19	11—27	...	Negative.

None of the controls of the various series developed leaf-curl disease.

TABLE III-B

Particulars of experiments performed to transmit the disease from sann-hemp to sann-hemp during 1937

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks.
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment, 2nd-3rd September 1937	1	...	2nd-3rd Sept.1937 —NOON.	20	2	71	Positive ; type S. V. I.
Age of diseased sann-hemp (Source) : 12 weeks.	2	...	Do.	20	3	79	Ditto
Age of healthy sann-hemp infected : 9 weeks.	3—8	...	Do.	20	2—5	...	Negative.
Series ii							
Time of experiment, and other details are same as in series *i*.	1	...	Do.	20	3	67	Positive ; type S. V. 2.
	2	...	Do.	20	3	72	Ditto.
	3—9	...	Do.	20	2—6	...	Negative.

None of the controls in the various series developed leaf-curl disease

TABLE IV-A

Particulars of experiments performed to transmit the disease from tobacco to tobacco during 1936

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks.
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment 14th-29th September 1936	1—6	*C*	14th-19th Sept. 1936—4 P.M.—8 A.M.	16—48	5—12	...	Negative.
Age of diseased tobacco (Source) : 14—16 weeks.	7	*C*	19th Sept. 1936—5 P.M.	48	5	39	Positive ; type *C*.
	8	*C*	Do.	48	3	...	Negative.
	9—12	*X*	18th Sep. 1936—4-30 P. M.	16	1—2	..	Do.
Age of healthy tobacco infected : 9—11 weeks.	12—13	*X*	28th Sept. 1936—5 P.M.	17	2	...	Do.
	14	*X*	Do.	17	3	31	Positive ; type *X*.
	15—17	*B*	25th Sept. 1936—4 P.M.	19	5	...	Negative.
Series ii							
Time of experiment, 8th-15th October 1936	1—7	*C*	8th-13th Oct. 1936—4 P.M.—5 P.M.	8—24	3—6	...	Negative.
Age of diseased tobacco (Source) : 12—13 weeks.	8—12	*B*	8th-10th Oct. 1936—4 P.M.—5 P.M.	24	1—7	...	Do.
Age of healthy tobacco infected : 12—13 weeks.	13	**A*	15th Oct. 1936—8 A.M.	8	3	26	Positive ; type *X*. *Type A (Source) contained a little mixture of *C & D*.
	14—17	*A*	Do.	8—9	4—5	...	Negative.

TABLE IV-A—*contd.*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series iii							
Time of experiment, 16th-17th Oct. 1936	1	*X*	16th Oct. 1936—9 A.M.	7½	2	25	Positive; type *X*.
Age of diseased tobacco (Source): 9 weeks.	2—3	*X*	Do.	7½	3—5	...	Negative.
Age of healthy tobacco infected: 9 weeks.	4	*X*	17th Oct. 1936—9 A.M.	8	5	22	Positive; type *X*.
Series iv							
Time of experiment, 28th October-6th November 1936	1	*C*	28th Oct. 1936—10 A.M.	5	10	18	Positive; type *C*.
Age of diseased tobacco (Source): 15—16 weeks.	2—14	*C*	28th Oct. and 1st Nov. 1936—10 A.M.	5—24	3—12	...	Negative.
Age of healthy tobacco infected: 6 7 weeks.	15	*C*	31st Oct. 1936—1 P.M.	24	8	22	Positive; type *C* & *D*.
	16	*C*	Do.	24	4	22	Positive; type *C*.
	17	*C*	Do.	24	7	12	Positive; type *X*.
	18—21	*C*	Do.	24	3—6	...	Negative.
	22	*C*	Do.	24	5	37	Positive; type *C*.
	23—24	*C*	Do.	24	4—8	...	Negative.
	25	*B*	28th Oct. 1936—10 A.M.	5	12	...	Do.
	26	*B*	31st Oct. 1936—1 P.M.	24	11	22	Positive; type *B*.

27—33	*B*	Do.	24	3—13	...	Negative.
34	*B*	1st Nov. 1936—3 P.M.	24	9	101	Positive ; A type *X*
35	*B*	Do.	24	11	110	Positive ; type *B*.
36	*B*	Do.	24	6	36	Positive ; *A* type *B* or *C & D*.
37	*B*	Do.	24	8	...	Negative.
38	*B*	Do.	24	7	20	Positive ; type *B*.
39	*B*	Do.	24	11	...	Negative.
40	*B*	Do.	24	9	20	Positive ; type *B*.
41	*X* of *C*	31st Oct. 1936—1 P.M.	24	4	21	Positive ; type *C & D*.
42	*X* of *C*	Do.	24	7	32	Positive ; *C* type *X*.
43	*A* with a little of *C & D*.	Do.	24	7	...	Negative.
44	Do.	Do.	24	9	22	Positive ; *C* type *X*.
45	Do.	1st Nov. 1936—3 P.M.	24	9	20	Positive ; type *X*.
46	Do.	Do.	24	13	102	Positive ; *C* type *X*.
47	Do.	Do.	24	7	...	Negative.
48	*A+B+C+D*	5th Nov. 1936—5 P.M.	21	26	140	Positive ; type *X*.
49	Do.	Do.	21	28	...	Negative.
50	Do.	Do.	21	33	102	Positive ; type *X*.
51	Do.	Do.	21	29	...	Negative.

Experiment Nos. 48—51. The vectors were fed separately on diseased plants of individual types A, B, C & D and then transferred together to single healthy seedlings in each case. The result was a synthetic mixture *X*.

TABLE IV-A—*contd.*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series v							
Time of experiment, 14th-16th December 1936	1—8	*A*	14th-16th Dec. 1936 —10 A.M.—4 P.M.	6—24	3—16	...	Negative.
Age of diseased tobacco (Source) : 16 weeks.	9—12	*B*	14th-15th Dec. 1936 —10 A.M.—4-30 P.M.	6—7	5—11	...	Do.
Age of healthy tobacco infected : about 8 weeks.	13	*B*	15th Dec. 1936— 9-30 A.M.	7	7	52	Positive : type *B*.
	14—15	*B*	15th-16th Dec. 1936 —9-30 A.M.—4 P. M.	6—24	3—6	...	Negative.
	16	*B*	16th Dec. 1936— 10 A.M.	6	7	68	Positive ; type *B*.
	17—22	*C*	14th-16th Dec. 1936 —10 A.M.—9-30 A.M.	7—24	6—13	...	Negative.
	23	*C*	16th Dec. 1936— 10 A.M.	6	5	59	Positive : type *C*.
	24	*C*	Do.	6	8	55	Do.
	25—33	*D*	14th-16th Dec. 1936 —10 A.M.—4 P.M.	6—7	4—14	...	Negative.
Series vi							
Time of experiment, 25th December, 1936-5th January 1937.	1	*A*	25th Dec. 1936— 4 P.M.	18	5	94	Positive ; type not clear.
	2	*A*	Do.	18	6	31	Positive ; type *A* ?

Age of diseased tobacco (Source) : 16—17 weeks.	3—10	*A*	Do.	18	4—7	...	Negative.
Age of healthy tobacco infected : 6—8 weeks.	11—18	*C*	Do.	18	4—9	...	Do.
	19	*C*	Do.	18	6	47	Positive ; type *X*.
	20	*C*	Do.	18	3	...	Negative.
	21	*D*	26th Dec. 1936—10 A.M.	6	5	...	Do.
	22	*D*	Do.	6	4	94	Positive ; type not clear.
	23—30	*D*	Do.	6	4—9	...	Negative.
	31	*A*+*B*+*C*+*D*	27th Dec. 1936—9 A.M.	6	11	...	Do.
	32	*A*+*B*+*C*+*D*	Do.	6	13	52	Positive ; type *X*.
	33	*A*+*B*+*C*+*D*	Do.	6	21	37	Do.
	34	*A*+*B*+*C*+*D*	Do.	6	24	...	Negative.
	35	*A*+*B*+*C*+*D*	Do.	6	18	93	Positive ; type *X*.
	36—37	*A*+*B*+*C*+*D*	Do.	6	13—15	...	Negative.
	38—39	*A* + *C*	Do.	6	11—15	...	Do.
	40	*A* + *C*	Do.	6	13	36	Positive ; A type *X*.
	41	*A* + *D*	Do.	6	7	37	Positive ; type *X*.
	42	*A* + *D*	Do.	6	9	...	Negative.
	43	*A* + *D*	Do.	6	11	47	Positive ; type *X*.
	44—45	*B*	4th-5th Jan. 1937—2 P.M.	24	2—4	...	Negative.
	46	*B*	Do.	24	6	87	Positive ; type not clear.
	47—51	*B*	Do.	24	3—5	...	Negative.
	52—56	*C*	Do.	24	3—5	...	Do.
	57	*C*	Do.	24	6	31	Positive ; type *C*.
	58—59	*C*	Do.	24	4	...	Negative.
	60—64	*X*	4th Jan. 1937—2 P.M.	24	2—6	...	Do.
	65	*X*	Do.	24	8	37	Positive ; type *X*.
	66—67	*X*	Do.	24	4—6	...	Negative.

TABLE IV-A—*concld.*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series vii							
Time of experiment, 6th-9th February 1937	1	*A*	6th Feb. 1937—3 P.M.	18	9	17	Positive ; type *X*.
	2—15	*A*	Do.	18	4—11	17	Negative.
Age of diseased tobacco (Source) : 22 weeks.	16—19	*B*	Do.	18	5—12	17	Do.
Age of healthy tobacco infected : about 7 weeks.	20	*B*	Do.	18	8	37	Positive ; type *B*.
	21—24	*B*	Do.	18	6—9	...	Negative.
	25	*B*	Do.	18	7	18	Positive ; type *B*.
	26—27	*B*	Do.	18	9	...	Negative.
	28	*B*	Do.	18	8	19	Positive ; type *B*.
	29	*B*	Do.	18	9	26	Do.
	30	*B*	Do.	18	9	...	Negative.
	31—45	*C*	Do.	18	5—13	...	Do.
	46—50	*D*	Do.	18	7—11	...	Do.

51	*D*	Do. .	18	7	20	Positive ; type not clear.
52	*D*	Do. .	18	6	24	Do.
53	*D*	Do. .	18	4	...	Negative.
54	*D*	Do. .	18	9	20	Positive ; type *X*
55	*D*	Do. .	18	6	...	Negative.
56	*D*	Do. .	18	6	22	Positive ; type not clear.
57 63	*D*	Do.	18	7—11	...	Negative.
64	*D*	Do. .	18	8	34	Positive ; type not clear.
65	*D*	Do. .	18	6	...	Negative.
66	*X*	7th Feb. 1937—2 P.M.	24	6	19	Positive ; type *X*.
67	*X*	Do. .	24	7	...	Negative.
68	*X*	Do. .	24	4	23	Positive ; type *X*.
69	*X*	Do. .	24	9	...	Negative.
70	*X*	Do. .	24	8	25	Positive ; type *X*.
71—73	*X*	Do. .	24	5—9	...	Negative.
74	*X*	Do. .	24	7	20	Positive ; type *X*.
75—77	*X*	Do. .	24	5—7	...	Negative
78—79	*X*	8th-9th Feb. 1937—2 P.M.	24	6—7	...	Do.
80	*X*	Do. .	24	5	11	Positive ; type *X*.

None of the controls of the various series developed leaf-curl disease.

TABLE IV-B

Particulars of Experiments performed to transmit the disease from tobacco to tobacco during 1937

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment, 14th-15th October 1937	1	*D*	14th Oct. 1937—9 A.M.	7	4	34	Positive : type *X*.
Age of diseased tobacco (Source) : 13 weeks.	2	*D*	Do.	7	5	32	Do.
Age of healthy tocacco infected : about 9 weeks.	3—17	*D*	Do.	7—24	4—9	...	Negative.
	18—20	*X*	14th-15th Oct.1937—9 A.M.	24	4—6	...	Do.
Series ii							
Time of experiment, 5th-9th November 1937	1—2	*A*	5th Nov. 1937—4 P.M.	24	4—5	...	Do.
Age of diseased tobacco (Source) : 10 weeks.	3—6	*D*	Do.	18—24	5—10	...	Do.
Age of healthy tobacco infected : 8 weeks.	7—8	*X*	Do.	18—24	1—8	...	Do.
	9—10	*A* type *X*	Do.	18—24	5—7	...	Do.
	11—15	*A* & *C*	Do.	24	1—9	...	Do.

	16 18	*A & C*	6th November 1937—3-30 P.M.	17½	5 10	...	Do.
	19—21	*B*	Do.	17½	2—5	...	Do.
	22	*C*	Do.	17½	6	30	Positive; type trace of C. recovered.
	23—30	*C*	Do.	17½	2—10	...	Negative.
	31	*C*	8th-9th November 1937—11-15 A.M.	24	12	25	Positive; *C* type *X*.
	32	*C*	Do.	24	3	...	Negative.
	33—35	*B*	Do.	24	2—5	...	Do.
Series iii.							
Time of experiment, 29th November-1st December 1937	1—6	*C*	29th November 1937—NOON.	24	2—8	...	Do.
Age of diseased tobacco (Source): 13 weeks.	7—9	*B*	Do.	24	1—5		Do.
Age of healthy tobacco infected: 7 weeks.	10—13	*A*	Do.	24	2—4	...	Do.
	14 15	*X*	Do.	24	2—3	...	Do.
	16—21	*D*	29th November 1937—4 P.M.	24	1—5	...	Do.
	22—23	*X*	30th November-1st December 1937—4 P.M.	24	8—9	...	Do.
	24—25	*C*	Do.	24	5—6	...	Do.

None of the controls of the various series developed leaf-curl disease.

N 2

TABLE V

Particulars of Experiments performed to transmit the disease from Ageratum conyzoides *to tobacco during 1936 and 1937*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment, 16th-17th December 1936	1	...	16th-17th December 1936—3 P.M.	25	16	34	Positive ; type *D.*
Age of diseased *Ageratum* (Source) : not known, obtained from field.	2	...	Do.	25	6	33	Do.
Age of healthy tobacco infected, 9 weeks.	3—6	...	Do.	25	6—11	...	Negative.
Time of experiment, 5th-8th September 1937	1	...	5th Sept. 1937—2 P.M.	25	3	55	Positive ; type *D.*
Age of diseased *Ageratum* (Source) : not known, obtained from field.	2—6	...	Do.	25	2—4	...	Negative.
Age of healthy tocacco infected, 8 weeks.	7	...	6th Sept. 1937—11 A.M.	21	4	...	Do.
	8	...	Do.	21	9	16	Positive ; type *D.*
	9—12	...	Do.	21	3—5	...	Negative.
	13	...	Do.	21	7	54	Positive ; type *D.*
	14	...	Do.	21	4	54	Do.
	15	...	Do.	21	3	15	Do.
	16	...	Do.	21	7	25	Do.
	17—19	...	Do.	21	5—7	...	Negative.
	20	...	Do.	21	3	49	Positive ; type *D.*
	21	...	7th Sept. 1937—5 P.M.	16	7	24	Do.

		...	Do.	16	8	...	Negative.
	23	...	Do.	16	10	53	Positive ; type *D*
	24	...	Do.	16	14	...	Negative.
	25	...	7th-8th Sept. 1937 —5 P.M.	16	13	15	Positive ; type *D*
	26	...	Do.	16	5	24	Do.
	27	...	Do.	16	14	14	Do.
	28	...	Do.	16	16	66	Do.
	29	...	Do.	16	8	13	Do.
	30	...	Do.	16	11	83	Do.
	31	...	Do.	16	12	...	Negative.
	32	...	Do.	16	8	24	Positive; type *D*.
	33	...	Do.	16	5	66	Positive ; *D* type *X*.
	34	...	Do.	16	12	53	Positive ; type *D*.
	35	...	Do.	16	10	35	Do.
	36	...	Do.	16	7	24	Positive ; type *D* ? (Recovered).
	37	...	Do.	16	9	65	Positive ; type *D*.
	38	...	Do.	16	7	68	Do.
	39	...	Do.	16	7	64	Do.
	40	...	Do.	16	9	66	Do.
Series ii							
Time of experiment, 26-28th September 1937	1—3	...	26th Sept. 1937—10 A.M.	25	7—11	...	Negative.
Age of diseased *Ageratum* (Source) : not known, obtained from field.	4	...	Do.	25	6	15	Positive ; type *D*.
Age of healthy tobacco infected : 6 weeks.	5	...	Do.	25	11	15	Do.
	6—7	...	Do.	25	7—8	...	Negative.
	8	...	Do.	25	9	23	Positive ; type *D*.
	9—10	...	Do.	25	6—8	...	Negative.
	11	...	Do.	25	12	30	Positive ; type *D*.
	12—15	...	Do.	25	8—13	...	Negative.
	16	...	Do.	25	9	15	Positive ; type *D*.

TABLE V—*contd.*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series ii—contd.							
	17	...	26th Sept. 1937—10 A.M.	25	11	...	Negative.
	18	...	Do.	25	6	27	Positive; type *D*.
	19—20	...	Do.	25	7—11	...	Negative.
	21	...	27th-28th Sept. 1937—11 A.M.—7-30 A.M.	20½	10	24	Positive; type *D*.
	22—24	...	Do.	20½	13—21	...	Negative.
	25	...	Do.	20½	24	11	Positive; *D* type *X*.
	26—30	...	Do.	20½	14—26	...	Negative.
Series iii							
Time of experiment, 2nd-5th November 1937.	1	...	2nd Nov. 1937—4 P.M.	44	6	24	Positive; type *D*.
	2—7	...	Do.	44	5—8	...	Negative.
Age of diseased *Ageratum* (Source): 13 weeks.	8—14	...	4th-5th Nov. 1937.—NOON.	29	6—12	...	Do.
Age of healthy tobacco infected: 7 weeks.	15	...	Do.	29	10	33	Positive; type *D*.
	16—17	...	Do.	29	6—8	...	Negative.

	18	...	Do.	29	9	31	Positive ; type *D*.
	19	...	Do.	24	9	...	Negative.
	20	...	Do.	24	13	27	Positive ; type *D*.
Series iv							
Time of experiment, 22nd-26th November 1937.	1—2	...	22nd Nov. 1937—NOON.	24	4—7	...	Negative.
Age of diseased *Ageratum* (Source) : 16 weeks.	3	...	Do.	24	8	41	Positive ; type *D*.
Age of healthy tobacco infected : 6 weeks.	4		Do.	24	1	19	Do.
	5	...	Do.	24	3	32	Positive ; type *D* (Recovered).
	6	...	Do.	24	5	19	Positive ; type *D*.
	7	...	Do.	24	1	19	Do.
	8	...	Do.	24	5	30	Do.
	9	...	Do.	24	5	19	Do.
	10	...	22nd Nov. 1937—4 P.M.	24	1	...	Negative.
	11	...	Do.	24	5	22	Positive ; type *D* ? (Recovered).
	12	...	Do.	24	6	19	Positive ; type *D*.
	13	...	Do.	24	4	19	Do.
	14	...	23rd Nov. 1937—4 P.M.	24	2	...	Negative.
	15	...	Do.	24	4	49	Positive ; type *D*.
	16—18	...	Do.	24	4—7	...	Negative.
	19	...	Do.	24	4	26	Positive : type *D*.
	20	...	Do.	24	5	28	Do.
	21	...	24th—26th Nov. 1937—3 P.M.—9 A.M.	42	3	...	Negative.
	22	...	Do.	42	4	26	Positive ; type *D*.
	23—30	...	Do.	42	2—6	...	Negative.

None of the controls of the various series developed leaf-curl disease.

TABLE VI

Particulars of Experiments performed to transmit the disease from tobacco to Ageratum conyzoides *during 1937*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series :							
Time of experiment, 29th-30th October 1937.	1—9	D	29th-30th Oct. 1937—5 P.M.—9 A.M.	16	2—9	15—47 average.	Positive; type resembled that occurred in nature. Profuse branching resulted.
Age of diseased tobacco (Source): 9 weeks.	...	...	...	...	...	35	
Age of healthy *Ageratum* infected: 11 weeks.							

None of the controls of the series developed leaf-curl disease.

TABLE VII

Particulars of Experiments performed to transmit the disease from Ageratum conyzoides *to sann-hemp during 1937*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment, 2nd-5th Sept. 1937.	1	...	3rd Sept. 1937—11 A.M.	23	3	78	Positive; type S. V. 2 Side shoots also diseased.
Age of diseased *Ageratum* (Source): 11 weeks.	2—5	...	2nd-4th Sept. 1937—NOON—10 A.M.	20—23	2	...	Negative.
Age of healthy sann-hemp infected: 9 weeks.	6—13	...	3rd-5th Sept. 1937—4 P.M.—10 A.M.	18—23	2—7	...	Do.
	14	...	4th Sept. 1937—10 A.M.	18	5	72	Positive; type S. V. 2.
	15—18	....	Do.	18	2—4	...	Negative.

None of the controls of this series developed leaf-curl disease.

TABLE VIII

Particulars of Experiments performed to transmit the disease from sann-hemp to Ageratum conyzoides *during 1937*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i							
Time of experiment, 11th-12th Oct. 1937.	1	S. V. 1.	11th-12th Oct. 1937—3 P.M.	24	9	...	Negative.
Age of diseased sann-hemp (Source): 11 weeks.	2—10	S. V. 1	Do.	24	5—12	49—69 average. 59	Positive; type resembled that occurred in nature.
Age of healthy *Ageratum* infected : 11 weeks.	...	...	...	...	...	...	Profuse branching resulted.
Series ii							
	1—11	S. V. 2	11th-12th Oct. 1937—3 P.M.	24	5—11	38—100 Average. 63	Positive; type resembled that occurred in nature. Profuse branching resulted.

None of the controls of this series developed leaf-curl disease.

TABLE IX

Particulars of Experiments performed to transmit the disease from Ageratum conyzoides *to* Ageratum Conyzoides *during 1937*

Series	No. of experiment	Type of disease used as source	Date of putting white-flies on the diseased plant	Period for which the specimens remained on the diseased plant	No. of specimens transferred to healthy plant	Period between the time of infection and appearance of disease	Results and remarks
1	2	3	4	5	6	7	8
				(Hours)		(Days)	
Series i.							
Time of experiment, 10th-11th October 1937 and 29th-31st October 1937.	1—19	...	10th-11th Oct. 1937—11 A.M.	24	5—11	13—71 average 42.	Positive; type resembled that occurred in nature. Profuse branching resulted.
Age of diseased *Ageratum* (Source): 14 weeks.	20	...	Do.	24	9	...	Negative.
Age of healthy *Ageratum* infected: 11 weeks.	21—24	...	29th-31st Oct. 1937—5 P.M.	22—24	6—10	27—39 average 34.	Positive; type resembled that occurred in nature Profuse branching resulted.

None of the controls of this series developed leaf-curl disease.

THE PHYTOPHAGOUS CHALCIDOID, *EURYTOMA SAMSONOVI* VAS., A SERIOUS PEST OF APRICOT AND SOME OTHER FRUITS IN INDIA

BY

HEM SINGH PRUTHI, M.Sc., Ph. D. (Cantab.), F. N. I.

Imperial Entomologist, New Delhi

AND

*H. N. BATRA, B.Sc., Assoc. I. A. R. I.

(Received for publication on 31st August 1938)

(With Plate XI)

Introduction

Chalcidoidea are well known as important parasites of insect pests. The phytophagous or plant feeding habits of certain families of this group, although seriously doubted at one time, are now fully established and numerous species are reported to be serious pests of some important cereal crops and fruits in various parts of the world. The notorious jointworm of wheat, *Harmolita tritici* (Fitch.) is widely distributed in the United States of America and several European countries, while *Eurytoma amygdali* End. infests almond, plum and prunes in Cyprus, Syria, Palestine, Italy, Russia, etc. The whole family Agaonidae on account of their food plants are known as fig insects, while a large number of Torymidae and Eurytomidae infest seeds, grains and several cultivated grasses [Crossby, 1909]. Gahan [1922] listed all the phytophagous Chalcidoidea excepting fig insects, and the species enumerated by him belong to as many as six families. Since 1922 a large number of new phytophagous forms have been discovered in several parts of the world.

The species included under the Chalcidoid families referred to above have a variety of feeding habits. Some genera like *Eurytoma*, *Tetrastichus*, etc., include both parasitic and phytophagous species [Phillips, 1927 ; Ishii, 1931]. There are some species which in the early stages of their development attack other insects and are thus parasitic, and complete the rest of their life-cycles on plant tissues [Phillips, 1927 ; Puzanov-Malyschev, 1936].

In India, besides the fig insects, only two phytophagous Chalcidoids are known to exist, *viz.*, *Eurytoma samsonovi* Vasiljev, which attacks apricot, peach, etc., in the North-West Frontier Province, and *Bruchophagus mellipes*

*The work of Mr. Batra incorporated in this paper was done by him when he was a post-graduate student in Entomology at the Imperia Agricultural Research Institute.

Gohar, feeding on *agathi* and *daincha* (*Sesbania aegyptica*) in Bihar and South India. Of these, *E. samsonovi* is the most important and the history of its discovery is rather long and interesting and is described below :—

Discovery of *Eurytoma samsonovi* in India and a review of allied pests in other parts of the world

In May 1910, Mr. Robertson Brown, Agricultural Officer in the North-West Frontier Province, sent a consignment of infested green apricots to the Imperial Entomologist for the identification of the pest concerned and suggestions for its control. Soon after the pest larvae were taken out of the fruits in the Insectary at Pusa, they died and at the request of the Imperial Entomologist another consignment was received in 1911. The material was carefully reared and a number of adult Chalcidoid wasps emerged between the 25th March and the middle of April. The specimens belonged to the family Eurytomidae, but as very few phytophagous Eurytomids were known at that time, they were taken to be the parasites of the real pest, and search for ' the pest ' continued. Infested fruits were kept under observation at Pusa during 1915-16, and again in 1926-27, but nothing except the Eurytomid emerged. This and the fact that by that time several Eurytomid pests had been discovered in other parts of the world gave rise to the idea that perhaps the so-called parasite was the real pest and therefore the Imperial Entomologist sent one of his assistants to the North-West Frontier Province to investigate the biology of the Chalcidoid at the spot. As a result of this investigation it was found that the Eurytomid itself was the real pest and the specimens were subsequently identified through the courtesy of Mr. Howard of the United States of America as *Eurytoma samsonovi* Vas. This species was originally described from Central Asia in 1915 where it is a serious pest of apricots, some plums and prunes, etc. [Vasiljev, 1915].

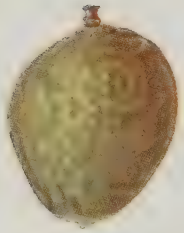
During 1928 the pest was studied in some detail in the Insectary at Pusa and a brief account of the work was published in the Report of the Imperial Entomologist for 1928-29 [Fletcher, 1930]. In 1934 when the junior author (H. N. B.) was working as a post-graduate student at Pusa, he visited the North-West Frontier Province and took observations on this pest in the field. In 1937 the Imperial Entomologist himself studied the pest in the field and in the spring of 1938 sent his assistant, Mr. H. L. Bhatia, to make observations on some points that were still obscure. The results of the studies carried out during different periods since 1910 are summarised in the following pages.

Before describing *Eurytoma samsonovi*, a brief account of the pests belonging to genus *Eurytoma* and to the other Indian genus, *Bruchophagus*, which occur in other countries may be given. Several species of *Eurytoma* occur in different parts of the world and attack several kinds of fruits, seeds, stems of plants, etc. Their life and seasonal histories in general are almost similar. The more important pests are listed below :—

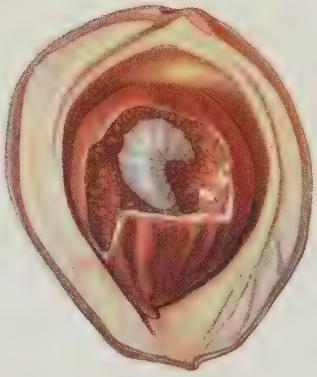
1

2

3

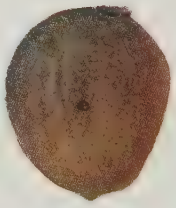


6



5

4



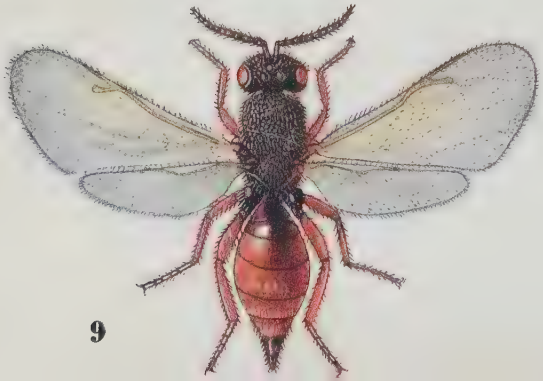
7

10

8

11a

11b



9

11

PLATE XI

Eurytoma samsonori Vas.

FIG. 1. Young grub 4-5 days old (×6)

2. Full-grown grub (×6)

3. Mouth parts of the same, note the powerful mandibles (×32)

4. Section of apricot fruit, showing the seed, the kernel of which has been eaten out by the grub but whose covering (testa) is still intact (×1)

5. Same, with the testa ruptured, showing the grub inside (×2)

6. Infested apricot fruit (×1)

7. Same, out of which the adult Chalcidoid has emerged by boring a hole (×1)

8. & 9. Pupa and adult female (×6)

10 & 11. Pupa and adult male (×6)

11*a-b*. Antenna and mandible of the male (×40)

Eurytoma amygdali End.—A very serious pest of almonds in Cyprus [Morris, 1930], Syria, Palestine [Aharoni, 1916], Bulgaria, Italy, and also a pest of apricots, plums and prunes in Russia [Puzanov-Malyschev, 1930; Vodinskaya, 1932].

E. orchidearum Westw.—A pest of orchids (*Cattleya* spp.) in Holland [Van Poeteren, 1932].

E. plotnikovi Niks.—Attacks *Pistacia* spp. in Russia [Nikol'skaya, 1935].

E. fellis Gir.—Causes galls in citrus stems in Queensland, New South Wales [Noble, 1933].

E. onobrychidis Niks.—Infests seeds of *Onobrychis sativa* in Russia [Nikol'skaya, 1933].

Bruchophagus gibbus Boh.—Infests clover seeds in Europe and America [Sorenson, 1935], lucern and alfalfa seeds in Russia [Ponomarenko, 1934; Nikol'skaya, 1932].

Seasonal history and nature of damage

The adult Chalcidoids appear in the orchards in the plains of the North-West Frontier Province during the second half of March, while at higher localities such as Parachinar their emergence is delayed up to the middle of April. At Haripur, in 1938, it made its appearance about the 20th March when the apricot fruits were yet of the size of small almonds. March 1938 was unusually wet and cold in that area and no copulating individuals were observed till the end of the month. Copulation generally took place while the individuals were flying in the air.

The exact method of oviposition has not yet been observed but it appears that the eggs are laid on the endocarp (stone) or inside the young seeds which is still tender. The female pierces the fruit with the help of its ovipositor. The young grubs on hatching work their way in and feed on the soft kernel of the seed. A parcel of raw apricots received from Haripur about the 20th April contained young grubs, 4 to 5 days old, feeding on the seed kernel. The young grub is translucent-white in colour and has a soft body but strong mouth parts (Plate XI, fig. 1). It voraciously feeds on the kernel leaving its coat in tact, which consequently presents a shrivelled and papery appearance (Plate XI, figs. 4 and 5). On account of the young seed having mostly been eaten away, the infested fruit looses its fresh green colour, becomes dark grey, shrivels up, and with even moderately strong breeze falls off the tree (Plate XI, fig. 6). Of about 1,200 fruits picked up from under the trees at Haripur during May 1938, about 85 per cent contained young grubs.

The fallen fruits get hardened and in many cases their pulps decompose, making the stones bare but the grubs continue to feed on the kernel inside the stones throughout the summer and are full grown in September and October. The fully fed grub (Plate XI, fig. 2) is very plump and fleshy and lies

curved up inside the seed. On the onset of winter the grubs go into hibernation and remain in this condition up to about the end of February when pupation takes place. The pupal period is two to three weeks and is also passed inside the seed. About the middle of March the adults emerge and leave the dry fruit by gnawing a clean hole through it (Plate XI, fig. 7). The cycle as outlined above is then repeated.

From the foregoing it will be noticed that the grub (larval) stage lasts for nine or ten months out of about one year in which a life-cycle is completed. Sometimes the grubs do not pupate next spring but pass the second summer and winter in the same stage. This happened at Pusa when the pest was being studied during 1916-17. In May 1937 a collection of about 450 infested fruits was obtained from Peshawar and Haripur and was kept in the Insectary at New Delhi. In the spring of 1938 while adults successfully emerged out of about 40 per cent and the grubs inside about the same number were found dead, in the remaining 20 per cent the grubs did not pupate and were still alive in August 1938. Probably they will pupate next spring. Thus, while normally a life-cycle takes about one year for completion, sometimes it extends over two years, most of the period being passed in the grub stage.

The seasonal history described above is roughly similar in broad outline to those of other Eurytomid pests which occur in Europe and America, though of course the actual times of the appearance of the adults in orchards and of hibernation of the grubs depend on the climate and weather conditions prevailing in different countries [Phillips and Poos, 1921 ; James, 1927 ; Puzanov-Malyschev, 1930 and 1936 ; etc.].

Description of various stages in the life-history

As already stated, the eggs of *Eurytoma samsonovi* have not yet been observed. The young grub (Plate XI, fig. 1), about four or five days old, is about 2·5 mm. long. It is thick in the middle and tapering towards the two ends of the body, the breadth in the thickest part being 0·75 mm. It is greyish in colour but the mouth parts are brown on account of heavier chitinization. The body is almost transparent, therefore, the course of the alimentary canal with its coloured contents can be easily made out. The grub invariably remains in a curved position, is legless and its head is always retracted inside the prothoracic region. The full-fed grub (Plate XI, fig. 2) is very large and plump, measuring about 7·5 × 3·3 mm., otherwise it does not differ in appearance from the young grub. Nine pairs of spiracles which are brown in colour and circular in outline, can be easily made out in the lateral sides of its body, which have also four to five rows of small spines or setae in the thoracic region and three to four rows in the region of the abdomen.

The pupae are dimorphic, the female pupa measuring 6·5 × 2·5 mm., being brown in the abdominal region and the male being smaller (5·5 × 2mm.) having almost a dark body (Plate XI, figs. 8 and 10). However, freshly formed pupae of both sexes are much brighter in colour. The adults

(Plate XI, figs. 9 and 11) like the pupae are dimorphic. The female is bigger (7·0×1·75 mm.) and much brighter in colour than the male, which measures about 5×1·5 mm. The eyes in both sexes are of beautiful red colour and the bodies are finely pubescent. The abdomen of the male is proportionately much smaller as compared to the thorax than it is in the female. The ocelli are almost in a straight line in the male, whereas they form a triangle in the case of the female. The antennae are geniculate as is usual in the Chalcidoidea, but are very different in the two sexes. The structure of the male antenna is almost similar to that of *Harmolita graminicola* Gir., described by James [1926]. Its scape (Plate XI, fig. 11*a*) is shorter and stouter than in the female. The funiculus consists of eight segments exclusive of a small ring-like joint between the first and second segments. The whole length of the funiculus is covered with sensony pits and setae or sensilli, the latter being longer and more numerous in the male than in the female. The mandibles (Plate XI, fig. 11*b*) are well developed, subtriangular in outline. Each mandible has a pair of sharp teeth close to which there is a pad-like chitinous ridge which has a crushing function.

Distribution, food plants and economic status

The most favourite host of *Eurytoma samsonovi* is apricot fruit. Peach and plum fruits are also attacked to some extent. The pest is widely distributed in the North-West Frontier Province including the hilly areas, *e.g.*, Parachinar, etc., but is serious in the plains only. It is especially bad in the Hazara district, the home of apricot fruits. In this locality, according to the report of the Director of Agriculture, as much as 70 per cent of the fruit gets attacked and falls off the trees. In the Haripur tehsil alone about 400 acres are under apricot orchards, containing about 70,000 trees. The average yield per tree is about two maunds, fetching about Rs. 5 per maund. The loss, even if only 50 per cent fruits fall off, works out at about four lakhs of rupees per annum. The extent of loss caused by the pest in the whole of the Province can therefore be easily imagined.

Several varieties of apricots are cultivated in the North-West Frontier Province and all of them are not equally susceptible to the attack of this pest. The *Kouri* variety which is the earliest to mature and fruits most profusely is most heavily attacked. Fortunately this variety is not highly priced. The latest variety, *jabhan*, which is the most delicious and sells at about Rs. 10 per maund suffers the least, about 10-20 per cent of the fruits being only affected. The other varieties, *bouei*, *sufeda* and *lallan* are intermediate in value as well as in the loss sustained.

It is of interest to record that one of us (H. S. P.) looked for this pest in the apricot orchards of Baluchistan in the summer of 1937, but found all localities free from it. There is also no report of the pest on any fruit in Kashmir, therefore its only habitat known at present is the North-West Frontier Province.

Control measures

In view of the nature of the life-history which indicates that the pest remains in grub stage inside the fallen fruits for about nine months in the year, the Imperial Entomologist suggested to the Agricultural Officer of the North-West Frontier Province that he should get all the fallen fruit collected and destroyed in some orchards and watch the incidence of the pest in the succeeding years. This method which does not involve much expenditure was tried by Mr. Robertson Brown in 1930 and found very effective.

After Mr. Robertson Brown's retirement, the control measure was tested on a large scale under the direction of Col. Noel, who stated in a letter to the Imperial Entomologist in 1935 " that the campaign had resulted in a very considerable reduction of the pest. " We understand that a fruit grower in the Haripur tehsil who had got only 45 mds. of fruit from his orchard in 1932, but had collected and destroyed all the fallen fruit during the summer of that year, got as much as 135 mds. from the same orchard next year.

In 1937, when the senior author visited Peshawar and met Col. Noel, the latter again spoke of the efficacy of the method and told him that he was moving his Government to introduce legislation to make it obligatory for owners of orchards to collect and destroy all fallen fruit under penalty of a fine.

It is interesting to record that in the case of Eurytomid pests in other countries also, *e.g.*, Cyprus, Russia, Queensland, etc., control measures almost similar to those described above are being advocated [Morris, 1930 ; Vodinskaya, 1932 ; Noble, 1933 ; etc.].

As regards natural enemies, Nikol'skaya [1932, 1933, 1935] has recorded several parasites of Eurytomid pests (*Eurytoma* spp., and *Bruchophagus gibbus*) in Russia, and Phillips and Poos [1921] have done the same in the case of the joint-worm of wheat in the United States of America, but so far there is no record of any parasite of *Eurytoma samsonovi* from India.

Summary

1. Several species of phytophagous Chalcidoidea occur as pests of wheat, figs, clover seeds, almonds, pistachio, orchids, etc., in various parts of the world.

2. *Eurytoma samsonovi* Vasiljev, which attacks apricot, peach, etc., in the North-West Frontier Province, was first bred out from infested apricots in 1911, but it was not till about sixteen years later that its exact status as a pest of apricot and other fruits in the province was determined.

3. Observations on the biology of this insect were made in the North-West Frontier Province, from time to time, since 1928. In the plains, the adults appear early in spring, copulate and the females oviposit inside the young seeds of apricot and other fruits. The grubs feed throughout the summer and hibernate in winter at the end of which pupation takes place. A proportion of the grubs fail to pupate and continue as grubs through the

whole of the next summer and winter, prolonging the life-cycle to two or three years. Short descriptions of the young and full-fed larvae, pupa and adult are given.

4. Apricot fruit is the most favourite host of *E. samsonovi* which though also found in the hilly areas of the North-West Frontier Province is serious pest only in the plains.

5. On the recommendation of the Imperial Entomologist, collecting and burning of all fallen fruit in the orchards was tried on a large scale as a method of control, in the North-West Frontier Province, and found very effective.

REFERENCES

*Aharoni, Y. (1916). *Intern. Rev. Sci. Proc. Agric. Mon. Bull. Agria. Intell. & Plant Dis. Rome* **7** (11)

Crossby, C. (1909). *Cornell Univ. Agri. Expt. Stn. Bull. No.* **265**, 368

Fletcher, T. B. (1930). *Sci. Rep. of Impl. Agric. Res. Inst.* (Pusa), p. 71

Gahan, A. B. (1922). *Proc. Ent. Soc. Wash.* **24**, 33-38

*Ishii, T. (1931). *Kontyu* **5**, 132-138

James, H. C. (1926). *Proc. Zoo. Soc. Lond.*, Pt. I, 75-182

———— (1927). *Ann. Appl. Biol.* **14**, 132-149

Morris, H. M. (1930). *Ann. Rept. Dept. Agric.* (*Cyprus*) *1929*, pp. 47-55

Nielson, J. C. (1906). *Zeit. Wiss. Insekten Biol.* **2**, 44-47

Nikol'skaya, M. N. (1932). *Plant Prot.* (I), 107-111

———— (1933). *Rev. Ent. U S S R.* **25**, 119-133

———— (1935). *Plan. Prot. Fasc.* **1**, 81-87

Noble, N. S. (1933). *Agric. Gaz.* (*New South Wales*) **44**, 465-469

Phillips, W. J. and Poos, F. W. (1921). *J. Agric. Res.* (*Wash.*) **21**, 405-426

Phillips, W. J. (1927). *Jour. Agr. Res.* (*Wash.*) **34**, 743-758

*Ponomarenko, D. A. (1934). *Grain Prod. J. Saratov* **3**, 34-46

Puzanov-Malyschev, E. W. (1936). *Zeitschr. Angew. Ent.* (*Berlin*) **22**, 631-642

———— (1930). *Rev. Russe Ent.* **24**, 166-178

*Sorenson, C. J. (1935). *Proc. Utah Acad. Sci.* **11**, 241-244

*Van Poeteren, N. (1932). *Versl. Plantenziektenk Dienst.* **66**, 134; *Agric. Expt. Stn. Bull. No.* **308**, 12

*Vasiljev, J. V. (1915). *Mem. Bur. Ent. Sci. Commit. Central Board and Administ. Agric.* (*Petrograd*) **11**, 1-19

Vodinskaya, K. I. (1932). *Plant Prot. No.* **1**, 97-106

*The references were seen in abstract only.

*STUDIES IN THE PERIODIC PARTIAL FAILURES OF AMERICAN COTTON IN THE PUNJAB

I. EARLY BREAKDOWN OF THE PHOTOSYNTHETIC APPARATUS

BY

R. H. DASTUR

Plant Physiologist, Punjab Agricultural College, Lyallpur

(Formerly Professor of Botany, Royal Institute of Science, Bombay)

[(Received for publication on 23rd August 1938)

(With Plates XII-XIV)

Introduction

In a preliminary note an early breakdown of the chloroplasts in the green leaves was described as an abnormal feature of the American cotton plants in the Punjab [Dastur, 1936]. At that time the different stages of the disintegrating process were not traced and the causes that give rise to the early breakdown of the chloroplasts were not investigated. This abnormal feature of apparently healthy leaves of the 4F cotton plants has now been studied during the cotton seasons of 1936 and 1937 and the important facts which have been discovered are summarised below :—

Chloroplasts are known to be stable protoplasmic bodies, which normally do not undergo disintegration in the functioning leaves, except under special conditions. The disintegration of the chloroplasts is reported to occur in plants suffering from bacterial [Smith, 1924] or virus diseases [Sorokin, 1927 ; Eckerson, 1926] and in plants attacked with insects [Painter, 1930]. No instance is recorded where the chloroplasts are found to disintegrate on account of some physiological disturbance in the plant. It is possible that such breakdown of the chloroplasts, as is found in the cotton plant, occurs in other plants as well and has merely escaped notice of the investigators.

Methods

The methods adopted to trace the different stages of disintegration were simple. Leaves of different ages were collected at regular intervals during the cotton season and were fixed in Flemming's weaker solution or Farmer's fluid

* This work was done in the Punjab Physiological (Cotton Failure) Scheme submitted by the Punjab Government and financed by the Indian Central Cotton Committee, Bombay.

or 4 per cent formalin. Sections of leaves were cut mostly by hand but sometimes by microtome. It is necessary to fix the leaves as an examination of unfixed material will lead to erroneous conclusions. Of various stains tried, haematoxylin and methyl violet gave the best results. Amongst the combination stains the Stoughton method was found most satisfactory. Though many varieties of exotic and indigenous cotton plants were examined, the following facts relate only to the 4F (*G. hirsutum*) cotton plant.

Investigation

The breakdown of chloroplasts and consequently of the photosynthetic system is found to occur in two ways, arising from two different causes. The two types of breakdown are classed as (A) mechanical and (B) chemical, and they are separately dealt with.

A. Mechanical breakdown of the chloroplasts

This type of breakdown of the chloroplasts is always preceded by a gradual accumulation of starch inside them. Normally starch does not accumulate inside the chloroplasts, as the starch that is stored up in them during the day is dissolved and removed at night. The same normal process is found to occur in the leaves of the cotton plant during the early part of the season. Towards the end of July and later leaves fully packed with starch begin to appear. The leaves collected at dawn are found to be full of starch. This indicates that dissolution and the removal of starch does not occur at night, though it continues to be formed and stored up in the chloroplasts during the day. The starch grains are found to grow bigger and thus increase in size on account of the fresh deposition of starch layers during the day.

A microscopic examination of such a leaf shows the distended palisade and spongy cells packed fully with unusually big starch grains which are joined laterally with one another, leaving no interspaces between them (Plate XII, figs. 3, 4, 5 ; Plate XIV, fig. 1). The chloroplasts in the cells are no longer distinguishable and are reduced to thin films surrounding the starch grains. The green colouring matter of the chloroplasts has come out in the form of small globules and covers the starch grains. The chloroplasts have bulged out and fused with one another. The palisade cells present the appearance of a reticulate network with starch grains occupying the meshes of the network and the bulged chloroplasts forming the net (Plate XII, figs. 3, 4). At many points the network of the chloroplasts is found broken due to the pressure of the starch grains. The starch grains in some cases get dissolved, leaving the broken fragments of the chloroplastic network in the cells. All such

stages of mechanical breakdown of the chloroplasts can be seen by suitable staining. The starch grains remain unstained while the chloroplasts are stained. Substances giving the reactions of tannins appear in the cell after the accumulation of starch has occurred (Plate XII, fig. 3 ; Plate XIV, fig. 1). The tannins first make their appearance on the lines of fusion of the starch grains and later cover them completely. The starch grains are so completely enveloped by tannins that starch-iodine reaction is not given by them, but the grains can still be distinguished by their size and shape. If the tannins and their derivatives are removed by suitable solvents the starch-iodine reaction is obtained at once. A palisade or a spongy cell may thus contain broken bits of chloroplasts, globules of green colouring matter, starch and tannins. The non-hydrolysis of starch very probably caused by some interference in the enzymic activities of the cell is thus responsible for the breakdown of the photosynthetic apparatus. Photosynthetic activity begins to decrease as starch remains unhydrolised and accumulates in the chloroplasts, and finally it comes to a standstill. The leaf continues to appear green and healthy so long as the globules of chlorophyll that have come out of the plastids remain undecomposed. Many such leaves with their load of starch and tannin or tannin alone are shed ultimately. In many cases the anthocyanin pigments develop in the epidermis prior to shedding.

B. Chemical breakdown of the chloroplasts

The second type of disintegration of chloroplasts is found to occur in young and half-matured leaves. The chloroplasts in the leaves of the cotton plant are round bodies which are stained uniformly by various dyes (Plate XII, fig. 1 ; Plate XIV, fig. 4). The first symptom of disintegration is the appearance of minute cavities in the chloroplasts (Plate XII, fig. 2 ; Plate XIV, fig. 4). In this type of breakdown the chloroplasts show swelling and vacuolation without starch accumulation. By further swelling of the chloroplasts the cavities enlarge and adjoining chloroplasts fuse together. They no longer appear as distinct bodies but become a sponge-like mass, filling the whole cell (Plate XII, fig. 2, Plate XIII, figs. 3, 4 ; Plate XIV, fig. 2). In the case of 4F Punjab-American cotton this type of disintegration can be seen with difficulty as the swelling is not very pronounced but in the case of Egyptian and Cambodia cotton plants all the stages of the process can be seen clearly (Plate XIII, figs. 3, 4). The swelling and ultimate dissolution of the chloroplasts is also accompanied by the formation of tannins in the cells (Plate XIII, fig. 4 ; Plate XIV, fig. 2). The chloroplasts become covered with tannins and can still be distinguished by the circular cavities.

In some leaves of the 4F and other cotton plants, small granules, one to three in number, appear in the body of the chloroplasts prior to the formation of circular cavities (Plate XIII, figs. 1, 2 ; Plate XIV, fig. 4). These granules are stained by methyl violet and other dyes in properly fixed materials. When they come out of the chloroplasts they leave cavities in them similar to those described above. They can be mistaken for spores which they are not. In some cells these granules are very minute, while in other cases they are larger and could be spotted easily.

Zirkle [1926], in an extensive study of the chloroplasts in higher plants, has described the presence of two types of granules in them. He has shown that the granules of the smaller type are not a part of the chromatophores, as reported by previous workers, while the larger granules are small pores or vacuoles in the chloroplasts. The granules observed in the chloroplasts of the cotton leaves would seem to be of a different type from those described by Zirkle. They appear to be distinct bodies which leave definite cavities in the plastids when they come out.

This type of breakdown of the chloroplasts develops first in the half-matured leaves and then spreads to the young leaves. The swollen sponge-like mass of the chloroplasts and tannins is the chief feature of the second type of breakdown of the photosynthetic apparatus. Both these types of disintegration may occur in the leaves of the same plant. The time when the disintegration sets in varies from plant to plant and particularly from field to field. It develops early in plants that later show the external symptoms of the ' disease ', while such premature breakdown of the chloroplasts does not occur in normal plants. These relationships between the breakdown of the photosynthetic apparatus and the bad opening of the bolls can be seen best in an extreme case of bad opening of the bolls. The same remarks apply to cotton plants bearing well-opened bolls where no such breakdown of the photosynthetic system is visible. In order to study this relationship it is necessary to make a periodic examination of the leaves of different nodes on a large number of plants. It should then be possible to establish the relation between the time of appearance of this internal symptom of the ' disease ' and the bad opening of the boll.

Experimental reproduction of the breakdown of chloroplasts

The two types of disintegration of the chloroplasts were reproduced experimentally in normal leaves in the following manner :—

If the daily increase in the size of the starch grains in the body of the chloroplasts, caused by some natural interference in the hydrolytic process,

results in the mechanical burst up of the chloroplasts, the same result could be obtained by interfering with the normal transport of carbohydrates from the leaves to the other parts of the plant by such means as ringing the stem. Cotton plants which did not show any such breakdown of the chloroplasts were selected for the experiment. Half the number of plants were ringed and the remainder were kept as controls. The leaves from the ringed and control plants were examined periodically. At the end of one month after ringing the leaves of the ringed plants showed a heavy accumulation of starch with distended cells (Plate XIV, fig. 5). The chloroplasts formed a network which was ruptured at many points. The leaves of the controls did not show an accumulation of starch or the breakdown of the chloroplasts.

The second type of disintegration of chloroplasts came to notice first in the leaves of plants in pots which had been treated with sodium chloride in an attempt to reproduce the symptoms of the ' disease '. In order to confirm this observation normal plants growing in a good field were treated with sodium chloride at the rate of 1500 lbs. per acre. The leaves of these plants were examined before the application of the salt and were found to be normal. A week after the application, the leaves showed the initial stages of disintegration of the chloroplasts (Plate XIV, fig. 6). Small cavities first appeared. The leaves became thick and fleshy and internally the mesophyll cells were elongated and showed turgidity. The chloroplasts later became vacuolated and sponge like with circular cavities. Tannins were produced in the cells (Plate XIV, fig. 7).

The increase in the thickness of the leaves and their fleshiness due to an increased water content were caused very probably by the absorption of the chlorine ions. Similar changes in the leaves of tobacco plant treated with sodium chloride were noticed by Garner, McMurtrey, Bowling and Moss [1930] and Wilson [1933]. The effect of sodium chloride on the cotton plant is investigated at present and the results will be communicated in a later publication.

Summary

The different stages of the disintegration of the chloroplasts in 4F Punjab-American cotton plant (*G. hirsutum*) are described. Disintegration is found to occur in two ways, produced by two different causes. The two types are classed here as (A) mechanical, (B) chemical. The mechanical burst-up of chloroplasts is caused by the daily increase in the size of the starch grains which remain unhydrolised, due probably to some interference in the enzymic activity of the cell. Chloroplasts bulge out and form a network with the starch

grains in the meshes. They also become ruptured, due to the pressure exerted by the starch grains. Substances giving reactions of tannins are formed later in the cells. The appearance of minute pores marks the starting point of the chemical type of disintegration of the chloroplasts. They first swell and large cavities appear in them. They then fuse together forming a spongy mass in the cells. Tannins develop and cover the dissolving chloroplasts. In some cases small granules appear before the small cavities are seen in the chloroplasts.

When the granules come out, cavities are left in the chloroplasts. Both types of disintegration are reproduced experimentally in normal leaves. The mechanical rupture of the chloroplasts is reproduced by interfering with the normal transport of carbohydrates from the leaves by ringing the stem. Chemical disintegration of the choloroplasts is brought about by the application of sodium chloride to the soil.

The breakdown of the photosynthetic apparatus is found to occur prematurely in the leaves of plants that develop the external symptoms of the 'disease' under investigation while no such disintegration of chloroplasts is noticed in the leaves of normal plants.

Acknowledgments

Thanks of the author are due to Dr. T. G. Mason, F.R.S., for his suggestion to reproduce experimentally the mechanical rupture of the chloroplasts in normal leaves and to Mr. H. R. Stewart, Director of Agriculture, Punjab, for giving all facilities for work and for correcting the manuscript.

REFERENCES

Dastur, R. H. (1936). *Ind. J. Agric. Sci.* **6**, 377-95

Eckerson, S. (1926). *Bot. Gaz.* **81**, 202-09

Garner, W. W.; McMurtrey, J. E.; Bowling J. D.; Moss E. G. (1930). *J. Agric. Res.* **40**, 627

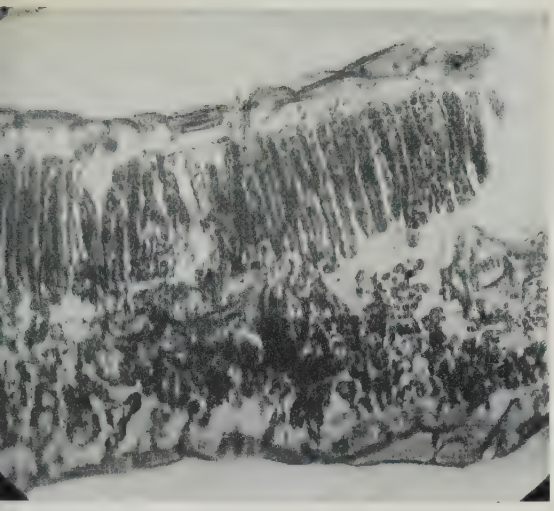
Painter, R. H. (1930). *J. Agric. Res.* **40**, 485

Smith, H. (1924). 'Bacterial Diseases of Plants'

Sorokin, H. (1927). *Phytopath.* **17**, 363

Wilson, L. B. (1933). *J. Agric. Res.* **46**, 819

Zirkle, C. (1926). *Amer. J. Bot.* **13**, 301



1. Section of a leaf of 4F (*G. hirsutum*) showing normal chloroplasts (×150)

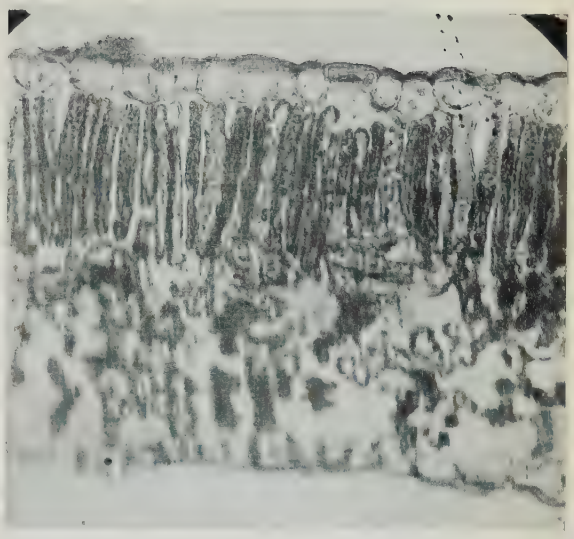
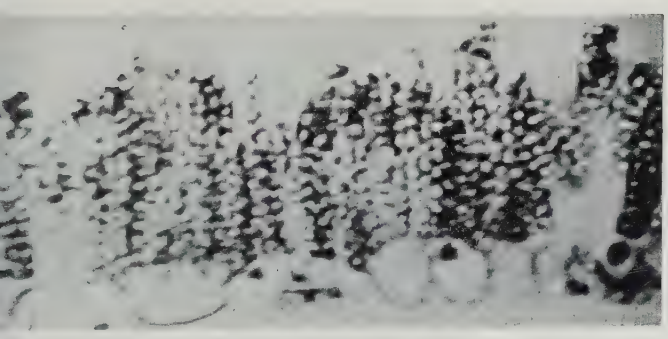
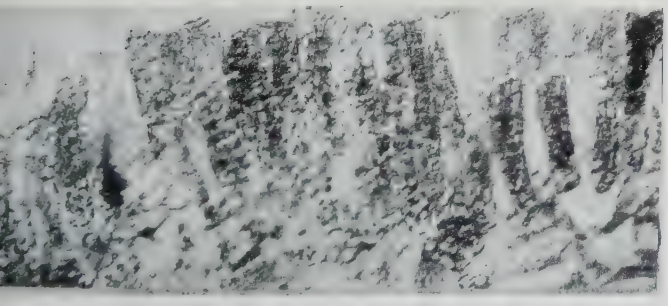


FIG. 2. Section of a leaf of 4F showing disintegrated chloroplasts with circular cavities (×150)



3. Section of a leaf of 4F showing large starch grains (unstained) inside chloroplasts which are joined with one another and have formed a network (stained)



4. The network of the chloroplasts after the removal of the starch grains by caustic potash (×350)

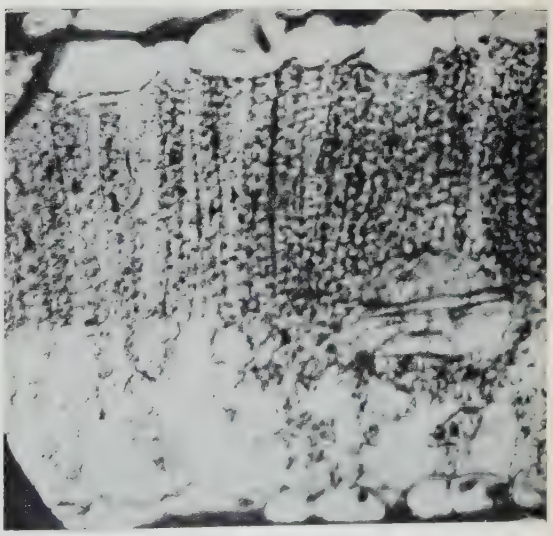
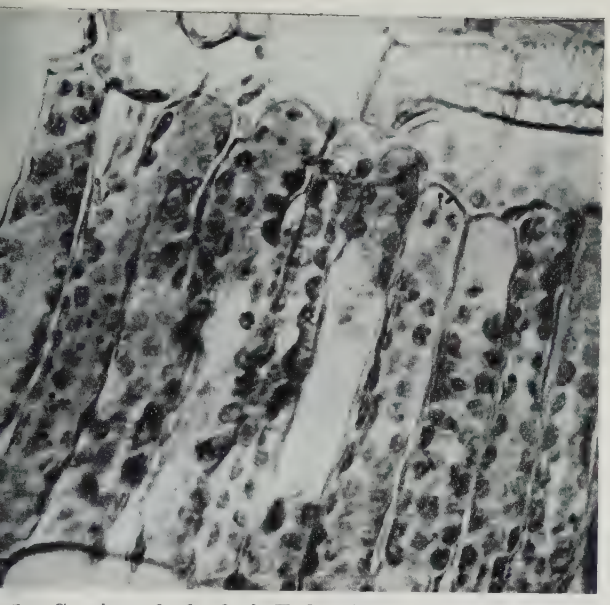


FIG. 5. Section of a leaf of 4F showing large starch grains inside chloroplasts (×400)



. 1. Section of a leaf of 4F showing small granules in the body of the chloroplasts (×620)

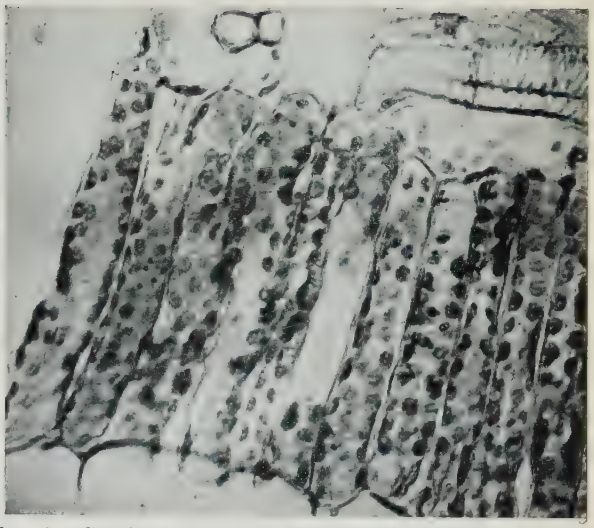
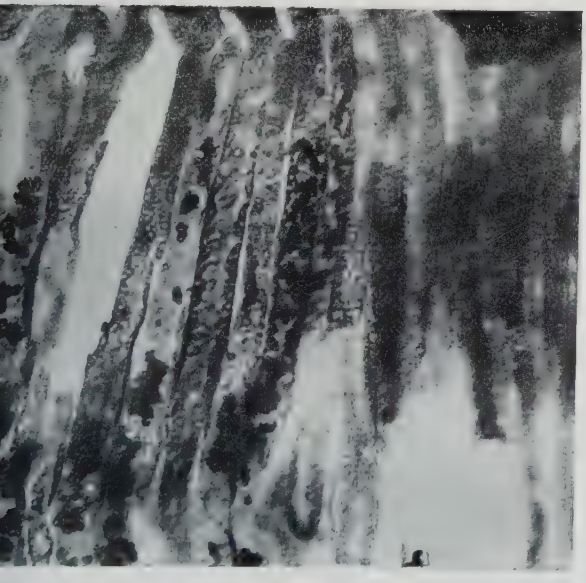


FIG. 2. Section of a leaf of 4F showing small granules in the body of the chloroplasts (×700)



3. Section of a leaf of an Egyptian cotton plant (Ashmoni), showing a swollen spongy mass of chloroplasts with large circular cavities (×500)

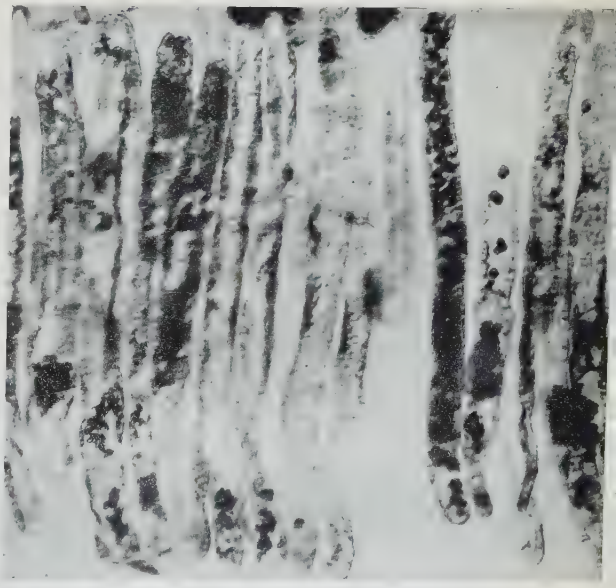


FIG. 4. Section of a leaf of an Egyptian cotton plant (Ashmoni), showing a swollen spongy mass of chloroplasts with large circular cavities (×600)

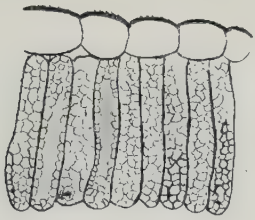


FIG. 1.

FIG. 2

FIG. 6.

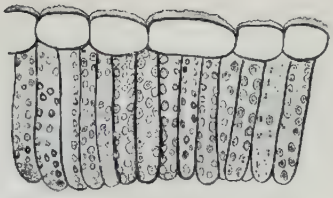


FIG. 4.

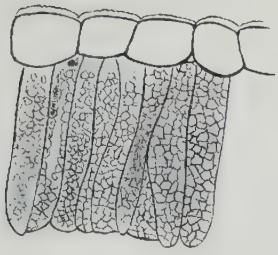


FIG. 5.

FIG. 3.

FIG. 7.

FIG. 1. Section of a leaf of 4F showing large starch grains surrounded by swollen chloroplasts, and tannin deposits (×200)

FIG. 2. Section of a leaf of a Cambodia cotton plant showing swollen sponge-like masses of chloroplasts with tannins (×250)

FIG. 3. Section of a leaf of 4F showing tannin deposits and the remains of disintegrated chloroplasts (×262)

FIG. 4. Section of a leaf of 4F showing small granules and cavities inside the chloroplasts (an early stage in disintegration) (×197)

FIG. 5. Section of a leaf of 4F showing accumulation of starch and tannins and the rupture of the chloroplasts produced by ringing the stem (×175)

FIG. 6. Section of a leaf of 4F treated with sodium chloride showing the early stage of chemical disintegration of chloroplasts (×232)

FIG. 7. Section of a leaf of 4F treated with sodium chloride showing swollen sponge-like masses of chloroplasts (×246)

II. FORMATION AND ACCUMULATION OF TANNINS IN LEAVES

BY

R. H. DASTUR

Plant Physiologist, Punjab Agricultural College, Lyallpur

(*Formerly Professor of Botany, Royal Institute of Science, Bombay*)

(Received for publication on 29th August 1938)

(With Plates XV-XVII)

Introduction

In a preliminary note [Dastur, 1936], certain deposits in the cells of the leaves and roots were reported to be one of the abnormal features of Punjab-American cotton plants that produced prematurely cracked bolls with immature seeds and poor lint. A study was, therefore, made of the development of these deposits in the cells, to determine their chemical nature and their exact relation with the ' physiological disease '.

Much work has been done on the chemistry of the cotton plant (*G. hirsutum* aud *G. herbaceum*) and especially on the chemistry of the natural organic colouring matter of the flowers, leaves and the bark. Perkin [1899, 1909, 1916] extracted and isolated various organic compounds of the flavonoal group from the flowers. They were found to be glucosides of quercetin and gossypetrin. Quercimeritrin, isoquercitrin and quercitrin also were obtained from the leaves by Perkin [1909] and by Viehoever, Chernoff and Johns [1918]. According to Standford and Viehoever [1918] these glucosides are present in the internal resinous glands that are distributed in the petals, leaves and bark of the root and stem. These internal glands are found to be present in all parts of the 4F cotton plant grown in the Punjab. They contain a yellow colouring matter giving the microchemical reactions of quercitrin and related substances. The same glands also give the reactions of gossypol, C_{30} H_{30} O_8, which was first described by Perkin [1905] and later by various workers like Reaves and Valle [1932]. On account of the presence of gossypol, the contents of these glands turn a cherry red colour when treated with strong sulphuric acid. They are found in the largest number in the roots of 4F and are distributed in the cortex, medullary rays and phloem.

Power and Chestnut [1926] in their extensive studies on the volatile and non-volatile constituents of the cotton plant have also isolated a large number of organic substances from the alcoholic extract of the leaves and flowers. The main object of their investigation and those of other workers was to

isolate the substance which was supposed to attract the cotton bollworm insect that causes such great damage to the crop but these attempts did not meet with any success.

The above review of the chemical investigations of the cotton plant makes no mention of the presence of tannins in it. It is possible that the tannin deposits described below were either absent in the plant parts extracted by these investigators or that they escaped isolation from the large number of complex organic substances that were present in such extracts. The cells containing these deposits first came to notice when the leaves and roots of the 4F plant were being examined under microscope. In leaves and roots preserved in 4 per cent formalin they could be seen distinctly. The cells containing these deposits differed from those which constituted the internal glands described above. These deposits gave all the microchemical reactions of tannins in the fresh materials of leaves and roots. Without these colour reactions they could not be detected in fresh leaves or roots as these deposits are colourless in the natural condition. Similarly, they cannot be seen in plant material which has been preserved in alcohol as they are soluble in alcohol and so are extracted by it.

Investigation

The cells containing these deposits are found in all parts of the plant. The deposits are found in the cells of the mesophyll, the conducting parenchyma, cortex, medullary rays, xylem and phloem parenchymatous tissues, the pith and the pericarp of the unripe fruit.

The tannin deposits are present in the form of solution (Plate XV, figs. 1-4). They are also present in cells that contain starch grains. The starch grains are either partly or completely covered by them (Plate XV, fig. 3). When completely covered by tannins the starch-iodine reaction is not obtained. In some cells each individual starch grain is covered by a layer of tannin and all the reactions of tannin are given by the grains (Plate XV, fig. 3). The tannin deposits are also present in cells that contain minute granules of calcium oxalate (Plate XV, fig. 1).

In formalin-preserved material of roots and leaves the tannins are precipitated in an insoluble form. As such they cannot be dissolved by any organic solvent and are rendered insoluble even in cold acids or alkalies. The task of extracting the substance of these deposits became easy, once the above facts regarding their solubility and other properties came to be known after a great deal of preliminary work.

The detailed methods of extraction, purification and quantitative estimation have been worked out and will be published in a separate paper. The deposits in the cells are made up of tannins which are soluble in water. They form dark brown coloured insoluble phlobaphenes on treatment with acids. They are also precipitated by formaldehyde in presence of acids. They give all the reactions of tannins as described by Russel [1935] and appear to belong to the class of tannins known as phlobatannins.

In addition to the tannin deposits soluble in water and alcohol the cells in the bark of the root of the diseased plants contain cell deposits that are insoluble in these solvents. This is not the case with the roots of healthy plants. The presence of these two different types of deposits came to notice when the soluble tannin deposits were removed by alcohol. The presence of these cell deposits in the roots of diseased plants and their absence in those of healthy plants became clear in the material extracted with alcohol. These deposits consist mainly of precipitated protein matter and the uncrystallised salts of organic acids, like oxalic and tannic acids. In this investigation only the tannin deposits that are soluble in alcohol and water will be considered.

Having established the chemical nature of these cell deposits, investigations were conducted to determine their relation to the disease under investigation. A thorough and periodic examination of the tissues of normal and diseased 4F cotton plants showed that the tannins were present in varying degrees in the roots of all plants but the case was different with the leaves. In diseased plants the formation and accumulation of tannins begins to occur at an early stage in the growth of the plant while leaves from the normal plants do not show any accumulation of tannins at any stage. Between these two extremes—the totally diseased and normal conditions—plants may be found in which tannins accumulate in the leaves to a less extent during the fruiting stage. It was noticed also that a slight accumulation of tannins occurred at the fruiting stage in young leaves but the same plant did not show any such accumulations at a later stage.

In the diseased condition of the plant the accumulation of tannins in the leaves begins early in the vegetative phase and continues till the end of the season. The accumulation of starch, disintegration of chloroplasts and accumulation of tannins are all associated together in the diseased plants. These internal symptoms indicate the onset of the physiologically diseased condition which manifests itself in the early shedding of leaves and the bad opening of bolls. The relation between these internal symptoms and the ultimate bad opening of the bolls requires to be systematically investigated and established. Preliminary work in that direction showed that in order to establish these relationships it was necessary to examine the leaves from different nodes from a number of plants for these symptoms at regular intervals from an early stage of growth up to the fruiting time.

Of the three internal symptoms the tannin deposits are the easiest to detect under microscope, and therefore the daily examination of the large number of leaves that is necessary in order to investigate the relation between the internal symptoms of the disease and the final bad opening of the bolls is facilitated. Attention is, therefore, focussed on the accumulation of tannins in the leaves, and its relation to the external symptoms of the disease is here investigated.

From previous records of yields and general observations made on the opening of the bolls by the staff of the Lyallpur Agricultural Farm, three fields, where 4F cotton was to be sown in the season of 1937, were selected for the detailed observations referred to above. These fields are situated in

squares 29, 27 and 32 of the Farm, and are designated good, medium, and bad according to the condition of the crop during previous years. The soils in squares 29, 27 and 32 are considered good, medium and bad respectively for cotton cultivation. In the beginning a fourth field in square 26 was also selected but the plot, where the plants were labelled for observation, showed suppressed growth from an early stage, owing to other adverse soil conditions and, therefore, it is not included here. It was noticed that extreme bad opening occurs in plants that show normal growth until September. The *p*H and the mechanical composition of the soils up to a depth of six feet are given in Table I. No attempt will be made here, however, to discuss the bearing of the soil conditions on the observations made on the internal and external symptoms of the disease.

TABLE I

pH and mechanical composition of the soils in the three fields

	Square 29			Square 27			Square 32		
	Clay	Silt	Sand	Clay	Silt	Sand	Clay	Silt	Sand
1st foot . . .	14·3	28·1	55·3	16·0	18·4	65·2	11·5	13·0	76·0
2nd foot . . .	18·0	26·8	50·3	16·8	16·1	65·3	13·7	17·9	65·2
3rd foot . . .	20·5	24·7	48·5	19·3	12·9	65·9	14·3	20·2	62·2
4th foot . . .	17·1	29·2	44·8	16·7	14·9	72·4	15·4	24·1	53·4
5th foot . . .	19·2	26·8	45·1	15·0	15·8	64·5	13·3	20·5	58·1
6th foot . . .	14·8	20·2	58·1	14·0	15·5	65·8	16·5	18·9	62·4
*p*H range in the six feet of soil.	8·11-8·26			8·09-8·18			8·07-8·34		

Silt = Silt + fine silt fractions. Sand = Coarse + fine sand fractions.

After final thinning of the plants in these fields was done, ten plants in each field were labelled at random in two adjacent rows and the leaves from different nodes from each plant were periodically examined. The accumulation of tannins was first noticed in the leaves from plants in the bad soil of square 32, towards the end of July. From that date onwards a systematic examination, for tannins, of the leaves from nodes 4, 8, 10 and 12, counting from the apex, from the ten plants in each of the three fields was made at intervals of about three weeks. When microscopic examinations of the leaves for tannins were made, it soon became evident that both the amount and the extent of the accumulation of tannins varied in different leaves of the same plant on the same date. It further differed in the leaves of the same node from the apex of the ten plants in the same field as well as from the plants of different fields on the same date of sampling. The concentration of tannins in the cells and the number of mesophyll cells that contained these deposits varied from leaf to leaf. In order to get some idea of the comparative degree and extent of accumulations of tannins in the different leaves from the same and different plants from the three fields it was necessary to fix, and use some standards for comparison. After some preliminary trials the following procedure was adopted.

Standard slides of sections of leaves showing different degrees and extent of tannin accumulation were prepared. Firstly, sections of leaves showing zero accumulation of tannins and those completely filled with thick deposits of tannins were obtained and mounted. Then sections of leaves showing accumulations of tannins varying between these two limits were prepared.

Table II gives a description of each standard slide with the respective number of + signs employed to indicate the tannin status of the leaves. The use of + signs is here made to express differences in the degree and the extent of accumulation of tannins in the leaves.

In addition to the standard slides described above other standard slides indicating the degree and extent of tannin accumulation between any two successive standards were also prepared and kept handy for comparison. The differences in the accumulation of tannins indicated by these standard slides are not quantitative but must be regarded as merely comparative. Four + signs do not indicate that the tannin accumulation is four times the amount indicated by one + sign. The different signs only indicate the different stages of tannin accumulations in the leaves as seen under microscope.

TABLE II

Description of standard slides with + signs employed to indicate the tannin status of the leaves

Sign used	Description of standard slide
Nil	All cells devoid of tannin deposits (Plate XVI, fig. 1).
+	Tannin deposits in the spongy cells only (Plate XVI, fig. 2).
++	Thin tannin deposits in a few palisade and spongy cells. The palisade cells are not completely filled with tannins (Plate XVI, figs. 3, 4).
+++	Thick tannin deposits in some palisade cells and a large number of spongy cells (Plate XVII, fig. 1).
++++	Thick tannin deposits in the majority of palisade cells (65 per cent) and spongy cells (Plate XVII, figs. 2, 3).
+++++	Thick tannin deposits in almost all cells (Plate XVII, fig. 4).

Small pieces cut from similar positions on the leaves, of the 4th, 8th, 10th and 12th nodes, from each of the ten plants in the three fields were preserved separately in 4 per cent formalin every three weeks. Sections from the leaves of the fourth node from the ten plants in one field were cut separately. Sections were cut at three points from the piece of preserved leaf. A large number was examined and the values of tannin accumulations were noted by comparison with the standard slides. The average of these values was taken as the tannin status of the 4th leaf on one plant. Such ten values for the tannin accumulations were obtained from the leaf of the fourth node of the ten plants and the mean of the ten values was taken to indicate the condition of the 4th leaf for tannins in a field. The same procedure was adopted for leaves from the remaining nodes of the ten plants in the same field and for the different leaves of the ten plants in the other two fields. Such observations of the tannin status of the leaves of the ten plants in these fields were done five times during the cotton season. The results obtained on the 29th September, 1937, for the four different leaves of the ten plants in each field are given separately in Table III, in order to give an idea of the variations in the tannin accumulation in the four different leaves of the ten plants in each field.

TABLE III

Values for tannin accumulation for the leaves of ten plants in the three fields on 29th September 1937

Plant No.	Square 29 (good soil) Node from the apex of the leaves				Square 27 (medium soil) Node from the apex of the leaves				Square 32 (bad soil) Node from the apex of the leaves			
	4th	8th	10th	12th	4th	8th	10th	12th	4th	8th	10th	12th
P1	++	±	Traces	+	++	*Nil*	*Nil*	*Nil*	+++++	+++++	Shed	Shed
P2	*Nil*	Traces	*Nil*	*Nil*	+++	+	*Nil*	+	++++	+++	Shed	Shed
P3	+	Traces	*Nil*	*Nil*	++	+±	+±	Shed	++++	++++	+++++	Shed
P4	*Nil*	Traces	*Nil*	*Nil*	+	*Nil*	*Nil*	Shed	+++++	+++++	Shed	Shed
P5	+	Traces	*Nil*	*Nil*	++±	*Nil*	±	Shed	+++	Shed	Shed	Shed
P6	*Nil*	Traces	*Nil*	*Nil*	++±	+±	Traces	+	+++++	+++++	+++++	Shed
P7	Traces	Traces	Traces	Traces	++±	+	Traces	+	+++++	+++++	+++++	Shed
P8	*Nil*	Traces	+	*Nil*	+	++	+++	++	+++++	Shed	Shed	Shed
P9	±	Traces	*Nil*	Traces	++	+	Traces	Shed	++++	++++	++++	Shed
P10	*Nil*	*Nil*	Traces	Shed	++	++	++	++	+++++	Shed	Shed	Shed

R

The accumulation of tannins is least in all the leaves of the ten plants in the good field while it is very high in the leaves of plants in the bad field. Slight amounts of tannins are found to accumulate in the leaves of plants growing in the medium field. The shedding of the leaves is also greatest in the plants from the bad field. It started from the middle of August onwards, while such shedding did not occur in plants in the good field.

The average values of tannins for the ten leaves of each node in the plants in the three fields for the five different dates are stated in Table IV. They represent the tannin status of the four different leaves of the plants on each date of sampling. (The shed leaves were not examined for tannins. In that case the average value of the number of leaves that remained on the ten plants is taken). The differences in the tannin accumulation in leaves of the three fields are obvious. In the case of the good field the tannins accumulate in the spongy cells of some leaves, while in the medium soil the accumulation occurs in some palisade cells as well. All the leaves of the bad field show large accumulations of tannins. These examinations of the leaves of the different nodes and at different dates in the three fields suggest that the tannin-like substances are produced in all cases but that they are used up probably as respiratory material, in the leaves of plants from the good field while they remain accumulated in the leaves of plants growing in the bad field.

Records of the total number of bolls produced by each of the ten plants were kept. The percentages of badly opened and well-opened bolls were also determined. A boll is regarded as badly opened when the majority of the seeds in the bolls is immature and the lint is weak. The weight of seed cotton per boll and the boll weights, *i.e.*, the number of bolls required to make one seer of seed cotton, were also determined. These results are stated in Table V. The averages of the ten plants are also given. There is no real bad opening of the bolls in the plants in the good field. The figures of badly opened bolls represent the stained bolls due to insect damage as in P_2 (Table V). Very few bolls opened badly in the case of plants in the medium soil while the percentage of badly opened bolls in the bad field varied from 73 to 100. The number of bolls produced per plant was also in the same order in the three fields. The weight of seed cotton per boll in the bad field was one fourth of that in the good soil, thus indicating the immaturity of seeds in the former. Consequently, the boll weights are very high in the bad field. The observations made on shedding of leaves showed that shedding began as early as August in these plants. All leaves up to the 10th node from the apex of one plant (P_{10}) were shed on the 23rd August, 1938, and up to the 12th node in another three plants (P_1, P_5 and P_9).

TABLE IV

Average values for tannin accumulations in the leaves of plants in good, medium and poor fields (each value is an average of ten leaves or less)

Date (1937)	Node of the leaf from the apex	Square 29 (good soil)	Square 27 (medium soil)	Square 32 (poor soil)
1st August	4th leaf	±	+±	+++
	8th „	Traces	+	+++
	10th „	Traces	+±	++
	12th „	Traces	+±	+
23rd „	4th „	Traces	+±	++++
	8th „	+	++	+++±
	10th „	+	++	+++
	12th „	+	±	+++±
7th Sept.	4th „	*Nil*	*Nil*	++++
	8th „	*Nil*	+	++++
	10th „	Traces	++±	+++++
	12th „	Traces	+	+++++
29th „	4th „	±	++	++++±
	8th „	Traces	+	++++
	10th „	Traces	+	++++±
	12th „	Traces	+	Shed
25th October	4th „	+	++	+++++
	8th „	Traces	±	+++++
	10th „	+	+	Shed
	12th „	Shed	Shed	Shed

TABLE V

Percentage of badly opened bolls, boll weights and number of bolls per plant in the three fields

	Total number of bolls			Percentage of badly opened bolls			Weight of seed cotton in grams per boll			Boll weights (number of bolls required to make one seer of seed cotton)		
	Good	Medium	Poor	Good	Medium	Poor	Good	Medium	Poor	Good	Medium	Poor
P1 . . .	64	75	13	1·5	18	84	3·5	1·5	0·65	257	600	1384
P2 . . .	91	132	43	16*	7	90	1·6	2·6	0·63	562	346	1451
P3 . . .	62	34	57	3	11	93	2·5	2·0	0·68	360	450	1323
P4 . . .	148	58	169	3	15	80	2·4	2·1	0·90	375	428	1000
P5 . . .	111	41	10	1	5	100	2·9	2·2	0·25	310	409	3600
P6 . . .	91	77	56	1	5	92	2·5	2·3	0·57	360	391	1578
P7 . . .	94	77	39	4	5	92	2·8	2·3	0·52	321	391	1726
P8 . .	140	28	14	2	21*	100	2·3	1·9	0·42	391	473	2143
P9 . . .	151	69	26	10	1·4	73	1·9	2·0	0·50	473	450	1800
P10 . . .	89	98	2	3	7	100	2·7	2·0	0·50	333	450	1800
Average for 10 plants	104	68·9	43	4·9	8·8	86	2·48	2·18	0·70	365	413	1289

*Most of these bolls are badly opened due to injury caused by the insects.

The results of tannin accumulations in the leaves of these plants in the bad field clearly indicate that they suffer from some physiological disorder which gives rise to bad opening and consequently low yields. This disorder is manifested in all the leaves by the presence of thick tannin deposits from August onwards. No such accumulations of tannins are noticed in the leaves from plants in the good field. Thus thick tannin deposits in the cells of the leaves can now be regarded as a definite internal symptom of the ' physiological disease ' of bad opening.

The total yields of the three fields were also recorded. They were about 21, 17 and 4 maunds of seed cotton per acre for the good, medium and the bad field, respectively.

The actual yield of plants in a field depends upon a number of factors and when the plants suffer from this ' physiological disease ', the yield varies according to the growth and vigour of plants in that field. All the ten plants in the bad field show accumulation of tannins to an equal extent as far as microscopic observations go, but the number of bolls produced by each plant and the yields obtained vary to a very great extent. The number of bolls produced varied from 2 to 169 and yield of seed cotton from 1 gram to 152 grams. The same holds good for plant in the good field, the yields varying from 150 to 360 grams. Thus the presence of tannins in the leaves from an early stage indicates that the plant is not normal and suffers from some physiological disturbance. Consequently, the yield given by that plant may be expected to be less than it would be in the absence of this disease.

It is recently found that the tannin-like substances found in the leaves of 4F plants are chemically of two types. The differences between the two types become apparent when the fresh leaves are extracted with water and tested for tannins. The one type described above, which is found in the leaves of ' diseased ' plants, gives blue colouration with osmic acid and a reddish brown precipitate with formaldehyde and hydrochloric acid. The second type does not give this reaction with osmic acid and gives a yellowish precipitate with formaldehyde and hydrochloric acid. The relationships found between the accumulation of tannins in leaves and the bad opening of the bolls discussed above refer to the first type only.

The second type of substance which also gets precipitated with formaldehyde in the cells of the leaves is found in plants which are growing on heavy soils. The growth of plants in such cases is suppressed, as was the case in the leaves of plants in square 26 referred to above, where the cells showed the deposits in the leaves preserved in formalin. In the beginning they could not be differentiated from the tannins of the first type but later the differences in their chemical nature came to be noticed. The chemical nature of this substance and its relation to the ' disease ' under investigation is investigated.

It may be mentioned that in normal and healthy plants the accumulation of any of these two substances in the cells of the leaves is not found to occur

while any derangement in the metabolic activities of the plant, it appears, leads to the accumulation of any one of the two types of substances in the leaves.

Conclusions

In extreme cases of the ' physiological disease ' of bad opening of the bolls tannins begin to accumulate in all the leaves of the plant from an early stage while no such accumulations occur in the leaves of healthy plants. Tannin accumulation in the leaves from an early stage of growth is, therefore, an internal index of the disease. The diseased plants do not show externally any symptoms of the disease during the vegetative phase and hence an early diagnosis of the onset of the disease is possible only by microscopic examination of the leaves of such plants.

The microscopic method of determining the diseased condition is both tedious and lengthy and it is not possible to make use of this method on a large scale. From a practical point of view it would be advantageous to use this plant index in finding out at an early stage the condition of the crop in a field. It is, therefore, necessary to replace the present microscopic method, which further is more or less qualitative and not quantitative, by a more rapid and easy one. It cannot be applied to determine the different degrees of intensity of the disease. A suitable method had to be devised by which different intensities of the diseased condition could be determined. A colorimetric method was, therefore, worked out and is being applied at present to test the relationship between this internal symptom and the bad opening of the bolls.

Summary

The chemical nature of the cell deposits in the leaves and roots of 4F Punjab-American cotton plants mentioned by the author in this Journal (Vol. 6, pp. 377-9, 1936) is determined. These deposits are made up of substances classed as tannins or their derivatives.

The accumulation of tannins giving the osmic acid test is found to occur in the leaves of plants which later develop the external symptoms of the disease, *viz.*, the reddening and shedding of the leaves and the bad opening of bolls. Such accumulation of tannins is not found to occur at any stage in leaves of normal plants.

The relationship between the accumulation of tannins in leaves and the final bad opening of bolls on the diseased plants is established by a systematic and detailed examination of different leaves of ten plants in three fields where the soils are classed as good, medium and bad, according to the condition of the cotton crop in previous years. The leaves of plants from the bad soil showed the largest accumulation of tannins from an early stage of growth. On an average the percentage of badly opened bolls on the plants was 85. The leaves from plants grown on the good soil showed no accumulation of

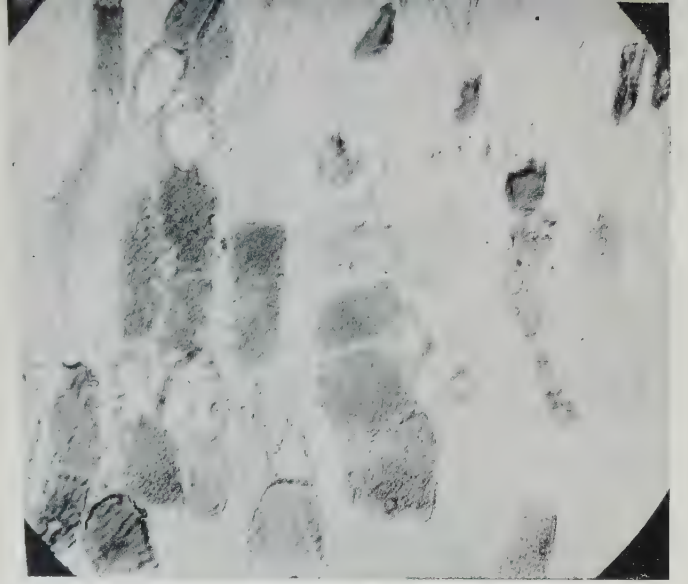


FIG. 1. Section of a root showing the cells of the bark containing tannin deposits

FIG. 2. Cells from the bark of a root of 4F showing tannin deposits (×260)

FIG. 3. Cells from the bark of a root of a 4F cotton plant showing starch grains covered with tannins (×400)

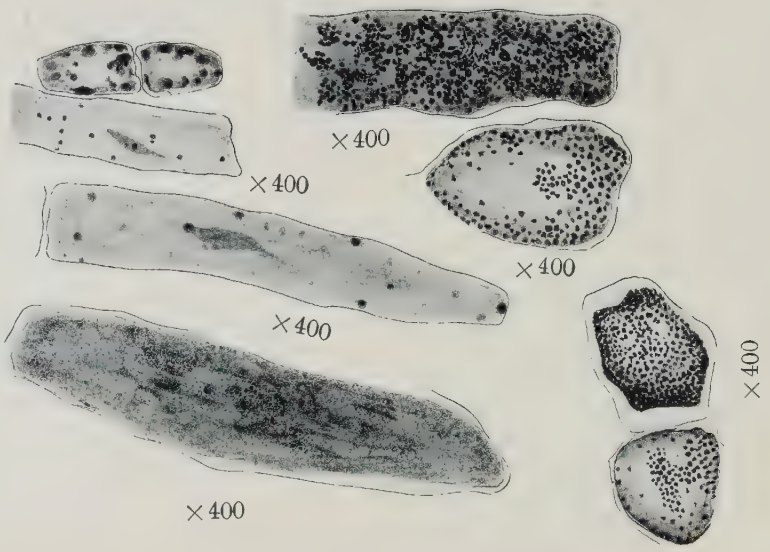


FIG. 4. Cells from the midrib of a leaf showing tannin deposits. Enlarged parenchymatous cells show the swollen spindle-shaped nuclei

PHOTOMICROGRAPHS OF STANDARD SLIDES FOR TANNIN ACCUMULATIONS

FIG. 1. Section of an old leaf of 4F cotton plant free from tannin (*nil*)

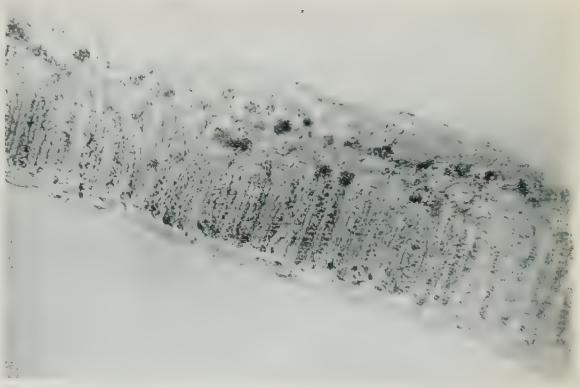


FIG. 2. Section of a leaf of a 4F cotton plant showing tannin deposits in the spongy cells (+)

IG. 3. Section of a leaf of a 4F plant, showing thin tannin deposits in a few palisade and spongy cells. The palisade cells are not completely filled with tannins (++)

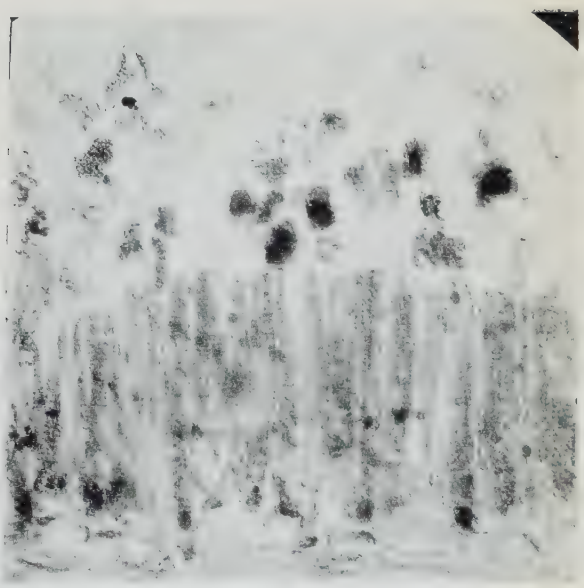


FIG. 4. FIG. 3 more highly magnified (++)

PHOTOMICROGRAPHS OF STANDARD SLIDES SHOWING TANNIN ACCUMULATION

FIG. 1. Section of a leaf of a 4F cotton plant, showing thick tannin deposits in some palisade and in a larger number of spongy cells (+++)

FIG. 2. Section of a leaf of a 4F plant showing thick tannin deposits in a majority (65 per cent) of palisade cells and spongy cells (+++)

FIG. 3. FIG. 2 more magnified (+++)

FIG. 4. Section of a leaf of a 4F cotton plant showing thick tannin deposits in almost all cells (++++)

tannins at any stage of growth and no bad opening of bolls occurred on these plants. The leaves from plants from the medium soil showed slight accumulation with about 10 per cent of bolls badly opened.

The yields of plants from the three fields were in the same order. It is important to note that the yield given by a plant showing this internal symptom of the disease depends also on other soil conditions.

The accumulation of tannins in leaves from an early stage can now be safely regarded as an index of an abnormal and unhealthy condition of the plant.

The differences in the tannin accumulation of the leaves given in this paper are roughly comparative. An attempt is now being made to determine these differences by a quantitative method and to find out whether any quantitative relation exists between the accumulation of tannins in leaves and the different degrees of bad opening noticed in different plants.

REFERENCES

Dastur, R. H. (1936). *Ind. J. Agri. Sc.* **6**, 377-9

Perkin, A. G. (1899). *J. Chem. Soc.* **75**, 825

——————— (1905). *J. Chem. Soc.* **87**, 107

——————— (1909). *J. Chem. Soc.* **95**, 825

——————— (1916). *J. Chem. Soc.* **109**, 145

Power, F. B. and Chestnut, V. K. (1926). *J. Amer. Chem. Soc.* **48**, 2721-37

Reaves, R. G. and Valle, C. C. (1932). *Bot. Gaz.* **93**, 259-77

Russel, A. (1935). *Chem. Rev.* **17**, 155-85

Standford, E. E. and Viehoever, A. (1918). *J. Agric. Res.* **13**, 419-35

Viehoever, A.; Chernoff, L. H. and Johns, C. O. (1918). *J. Agric. Res.* **13**, 345

VARIATIONS IN THE MEASURABLE CHARACTERS OF COTTON FIBRE

I. VARIATION WITH RESPECT TO THE LENGTH OF THE FIBRE

BY

R. L. N. IYENGAR, M.Sc.

Agricultural Research Institute, Coimbatore

(Received for publication on 26th November 1937)

(With eight text-figures)

CONTENTS

	Page
I. Introduction	305
II. Material and sampling	306
III. Experimental methods	306
IV. Discussion of results	311
(*a*) Immaturity	311
(*b*) Convolutions	317
(*c*) Maximum ribbon-width	320
(*d*) Minimum ribbon-width	321
(*e*) Mean ribbon-width	321
(*f*) Swollen fibre-diameter	323
(*g*) Wall-thickness	324
(*h*) Weight of 'dead' and thinwalled fibres	324
V. Conclusions	326
VI. Acknowledgments	326
References	327
Appendix	328

I. Introduction

It is a well-known fact that the individual fibres that go to make up a sample of cotton vary considerably in their physical properties. These variations are brought about by numerous causes both genetic and environmental. They have been dealt with in detail by Turner [1929], and others [Editorial, 1930]. In the present work and in the ones that are to follow, it is the object of the writer to study the extent of variation that is caused by each of the

different factors enumerated by Turner. The variation of the fibre-weight with respect to length was dealt with in a previous paper [Iyengar and Turner, 1930] and the present work deals with the variation of the other properties with respect to length.

II. Material and sampling

The material used in the present enquiry consisted of four samples of cotton, one belonging to strain Karunganni C 7 (*G. indicum*), another to strain 2405 (*G. herbaceum*) and the remaining two to strain Co 2 (*G. hirsutum*), the difference between the last two being, that one was collected from the irrigated and the other from the rain-fed plots of a randomised replicated experiment laid out for a study of the effects of irrigation.

The sampling was made with the object of having it as uniform as possible by eliminating the extraneous influences. The variation between samples of different plants was minimised by confining the sampling to as few plants as were barely necessary, the variation due to the age of the plant was minimised by taking the picking on a particular day, and that due to the composition of bolls and locks by taking only those bolls that were three locked and which contained the same number of seeds in each lock. All locks with one or more aborted seeds were discarded. The fourth seed of each lock alone was selected to form the sample.

III. Experimental methods

The seeds were carefully delinted by hand so as not to break any of the fibres and out of the lint obtained, a sliver was prepared by using the drawbox of the Balls Sorter equipment. After arranging the fibres from the sliver on a Baer Sorter, the fibres of different length-grades were extracted in the manner described in the previous paper [Iyengar and Turner, 1930]. A tuft of fibres containing approximately the required number of fibres of a particular length-grade was placed on a piece of plush and the fibres removed from them were used for the determination of the following properties :—

1. Number of convolutions per unit length of the fibre,
2. Ribbon-width—maximum, minimum and mean,
3. Swollen fibre-diameter,
4. Swollen wall-thickness, and
5. Immaturity.

Six consecutive length-classes differing by 1/8 in. namely, from 10/8 in. to 5/8 in., were examined in either of the samples of Co 2 and five (9/8 to 5/8 in.) in each of strains Karunganni C 7 and 2405.

Regarding the number of fibres to be tested for the determination of the different properties, Koshal and Turner [1930] have stated that it should be 300 for ribbon-width and 500 for convolutions. As the sample from which these numbers were derived was a bulk material, it was considered that in the present set of samples, where causes of variation were minimised and where the material under test consisted of a small tuft of fibres of uniform length, the variation would not be so high. Further the work being arduous, 200 fibres only were examined for each property. For the estimation of maturity, however, 200 more fibres were tested except for strain 2405*. The results obtained, however, indicate that the variability does not always decrease as anticipated, though in some cases the standard errors were small enough. The number of fibres tested should, therefore, be considered not quite adequate in all cases.

The fibres were mounted with seccotine in the manner usually followed for the determination of the convolutions and ribbon-width. Generally ten fibres were mounted on each slide taking care to have at least one of their ends nearly in a straight line, so that they may be covered with a coverslip later.

By moving the slide under the microscope (magnification $\times 260$) the length of the fibres and the total number of convolutions along the whole length were determined. Neither the direction of the convolutions nor the number of reversals was taken into account. The maximum ribbon-width and the minimum ribbon-width [Barritt, 1930] in the immediate neighbourhood of the maximum, were then determined at ten different places, spaced nearly equally apart along the length of the whole fibre. When all the fibres on the slide had been examined, they were fixed at the two ends by means of the wax used for fixing the fibres for strength determination and were covered with a suitable coverslip. The fibres were then irrigated with 18 per cent solution of caustic soda and examined half an hour † later under a higher magnification ($\times 590$).

It is well known that under this treatment a ripe fibre gets fully swollen and assumes a rod-like appearance. But an immature fibre shows a tubular structure with an internal canal. The thickness of the wall varies according to the degree of maturity of the fibre. The diameter of the fibre (D) and the diameter of the internal canal (d) were measured in the microscope at ten equal intervals along the length of the whole fibre, as was done in the case of

* As there was negligible immaturity in this strain, 200 fibres were deemed sufficient.

† Any amount of this resting period, above a minimum, was found to have no effect on the dimensions measured, under the magnification used, provided the solution did not evaporate to dryness.

the ribbon-width. D, then, represents the diameter of the fibre-cell and $D-d$, twice the wall-thickness of the swollen fibre.

The ratio of the fibre-diameter to wall-thickness of the swollen hair was used to estimate the maturity of the fibre. In order to determine the exact limits of the ratios between which fibres of the different maturity classes may be said to lie, the fibre was carefully scrutinised both in the raw and the swollen state and its condition of maturity, as to whether it can be classed as ripe, dead or thin-walled, was recorded.

It may be mentioned here that though most of the fibres generally exhibited uniformity in the degree of ripeness along their length, there were some fibres which showed wide variations *. Taking only those fibres that exhibited uniformity, a study of the distribution of the ratio of fibre-diameter to wall-thickness was made in the three maturity classes, in all the four cottons. The results obtained in the case of the rain-fed sample of Co 2 is recorded in Table I and Fig. 1 to serve as an example. The distributions in the other cottons also indicated similar limits of the ratio for the three maturity classes. Even in the strain 2405, where the immature fibres are very few, the same limits are found to be adhered to.

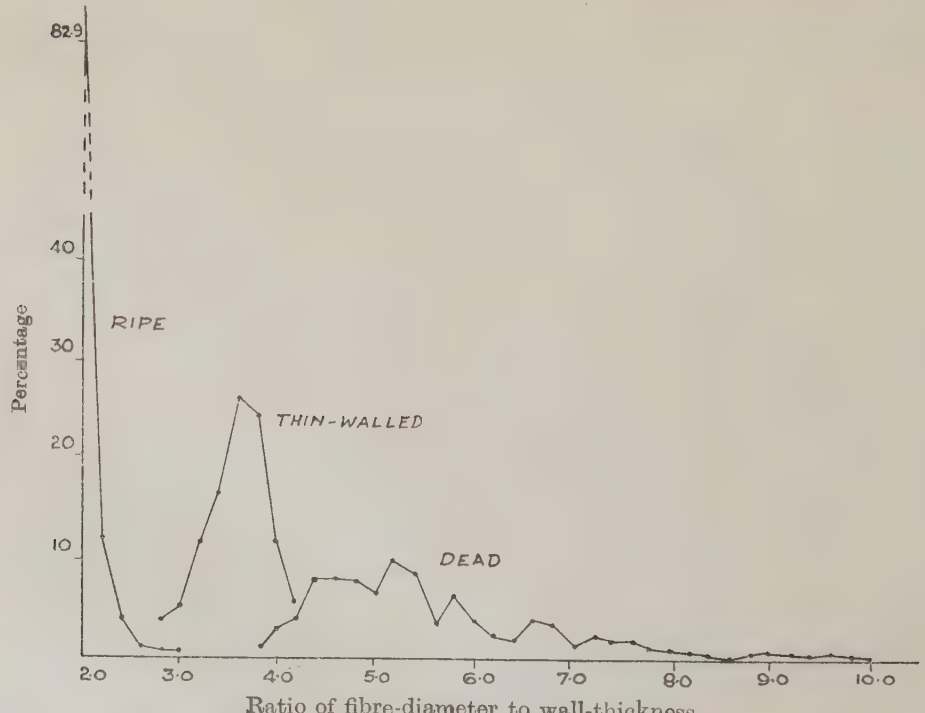


FIG. 1.

* Such a feature has also been noticed by Gulati and Ahmad [1935].

TABLE I

Ratio of fibre-diameter to wall-thickness

Ripe fibres			Thin-walled fibres			Dead-fibres		
Class interval	No.	Per-centage	Class interval	No.	Per-centage	Class interval	No.	Per-centage
2·00—2·10	417	82·9	2·71—2·90	2	3·2	3·71—3·90	2	0·6
2·11—2·30	60	11·9	2·91—3·10	3	4·8	3·91—4·10	9	2·8
2·31—2·50	17	3·4	3·11—3·30	7	11·3	4·11—4·30	17	5·4
2·51—2·70	5	1·0	3·31—3·50	10	16·2	4·31—4·50	23	7·3
2·71—2·90	2	0·4	3·51—3·70	16	25·8	4·51—4·70	24	7·6
2·91—3·10	2	0·4	3·71—3·90	15	24·2	4·71—4·90	23	7·3
			3·91—4·10	7	11·3	4·91—5·10	20	6·3
			4·11—4·30	2	3·2	5·11—5·30	31	9·8
						5·31—5·50	27	8·5
						5·51—5·70	10	3·2
						5·71—5·90	20	6·3
						5·91—6·10	12	3·8
						6·11—6·30	7	2·2
						6·31—6·50	6	1·9
						6·51—6·70	12	3·8
						6·71—6·90	10	3·2
						6·91—7·10	5	1·6
						7·11—7·30	6	1·9
						7·31—7·50	7	2·2
						7·51—7·70	6	1·9
						7·71—7·90	4	1·3
						7·91—8·10	3	0·9

TABLE I—*contd.*

Ripe fibres			Thin-walled fibres			Dead fibres		
Class interval	No.	Per-centage	Class interval	No.	Per-centage	Class interval	No.	Per-centage
						8·11—8·30	2	0·6
						8·31—8·50	1	0·3
						8·51—8·70	0	0·0
						8·71—8·90	1	0·3
						8·91—9·10	3	0·9
						9·11—9·30	2	0·6
						9·31—9·50	2	0·6
						9·51—9·70	1	0·3
						9·71—9·90	2	0·6
						9·91—10·10	1	0·3
						Greater than 10·10	17	5·4
Total .	503			62			316	

It will be seen that the ripe fibres range from 2·0 to 3·0; but most of the values lie in the neighbourhood of 2·0 and there is no value beyond 3·0. The dead fibres start at 3·8 and the value of the ratio rises to as high as 12. Only two fibres, or 0·6 per cent of the total number in this class, fall below 4·0. The thin-walled fibres lie between 3·0 and 4·0 with a little tail on either side and with mode at 3·6. Thus the ranges between which fibres of the three types of maturity lie seem to be circumscribed. The ripe fibres may be said to range from 2·0 to 3·0, the thin-walled fibres from 3·0 to 4·0 and the dead beyond 4·0. Employing these limits as standards, the percentages of the fibres in the different classes have been calculated.

Peirce and Lord [1934] have also considered this aspect and they have stated that " the limits of the thin-walled class are defined roughly by the

values 0·2—0·35 for the ratio of wall-thickness to ribbon-width " *which when converted into the ratio adopted in the present enquiry † work out to be 2·86 to 5·0. The lower limit is practically the same as the one found here, namely, 3·0 but the upper one is a little higher. But from a comparison of the ratios with the actual degree of maturity of the fibres, it was found, as shown in the foregoing distributions, that 4·0 was the more reliable limit in the samples handled here.

It may also be mentioned that these workers have used the cut section for their measurements. They have stated that they used " a method designed to secure a minimum of distortion or obliquity " but the error due to this cannot be as completely eliminated as in the present method, where it is non-existent. Moreover, the sections show the value at a particular portion of the fibre which may not be the representative behaviour along the entire length, as it is in the present case. These differences between the methods may possibly account for the higher value obtained by them for the upper limit of the thin-walled fibres, presuming that there has been no personal factor influencing the fixing of the border itself.

The significance of the differences between the maturity percentages has been tested by the use of the standard error, S, calculated according to the formula $S^2=\frac{pq}{n}$, where p and q represent the proportions of the fibres falling within and outside a class respectively, and n, the number of fibres examined [Yule, 1929].

IV. Discussion of results

Before taking up the discussion of the results proper, it may be stated that the normality or otherwise of any distribution, stated in the following discussion, is as judged visually from the frequency polygons only; no statistical tests for judging normality or otherwise have been made. The standard errors and the values of significance have been calculated wherever the distribution can be deemed nearly normal.

The variation of all the properties examined among the different length-grades may be seen at a glance in Table II.

(*a*) *Immaturity*

Before taking up the variations in the physical properties it would be appropriate to discuss here the variations in the maturity of fibres as this character is observed to influence almost all the other properties.

* From the statement " after separating the three groups of the immaturity test " it is assumed that these authors have used fibres swollen in caustic soda.

† As the present work was started in 1933, much earlier than the publication of the work of Peirce and Lord and as the ratio of fibre-diameter to wall-thickness was chosen from the commencement, it has been employed instead of the inverse ratio of wall-thickness to diameter used by these authors.

TABLE

Summary

Cotton	Length				Convolutions per cm.			Ribbon-width					
	Expected in		Actual mean in mm.	S. E.	All fibres	Ripe fibres only	S. E. of (7)	Maximum	S. E.	Minimum	S. E.	Mean	S. E.
	inch	mm.											
1	2	3	4	5	6	7	8	9	10	11	12	13	14
Co 2 Irrigated	10/8	31·8	30·6	0·148	26·4	43·5	1·38	20·5	0·185	8·6		14·7	0·092
	9/8	28·6	28·1	0·106	31·0	57·0	1·92	21·4	0·176	8·2		14·9	0·092
	8/8	25·4	25·6	0·131	43·2	63·7	1·61	21·3	0·199	9·1		14·9	0·106
	7/8	22·2	21·5	0·150	50·6	63·9	1·76	20·6	0·194	9·5		15·2	0·111
	6/8	19·1	17·6	0·163	47·0	66·8	1·98	21·4	0·240	9·0		15·3	0·143
	5/8	15·9	13·8	0·179	40·6	65·0	2·02	20 9	0·222	8·4		14·8	0·139
Co 2 Rain-fed	10/8	31·8	30·9	0·147	44·2	71·4	1·63	19·9	0·203	7·8		13·9	0·092
	9/8	28·6	28·6	0·118	44·3	74·5	2·37	20·8	0·194	7·5		14·2	0·092
	8/8	25·4	26·7	0·118	45·8	79·7	2·32	20·6	0·222	7·5		14·1	0·088
	7/8	22·2	22·6	0·144	59·8	79·6	2·15	20·8	0·203	8·7		14·9	0·106
	6/8	19·1	18·3	0·161	44·2	67·1	2·07	21·3	0·240	8·7		15·0	0·129
	5/8	15·9	14·9	0·185	48·4	70·2	2·76	21·7	0·264	7·6		15·0	0·143
Karunganni C 7.	9/8	28·6	28·5	0·115	38·8	46·8	1·32	17·2	0·139	8·3	0·111	13·3	0·078
	8/8	25·4	25·6	0·095	33·7	40·6	1·15	18·0	0·157	8·5	0·148	13·8	0·083
	7/8	22·2	22·8	0·106	31·6	38·9	1·10	19·1	0·217	8·6		14·3	0·088
	6/8	19·1	19·5	0·123	26·6	36·0	0·99	19·7	0·269	8·6		14·8	0·097
	5/8	15·9	15·4	0·156	20·0	31·2	1·06	21·1	0·287	8·3		15·3	0·092
Strain 2405	9/8	28·6	26·7	0·138	25·8	26·0	0·49	17·2	0·148	10·4	0·083	13·9	0·106
	8/8	25·4	24·7	0·086	31·7	32·3	0·58	17·6	0·129	10·7	0·079	14·2	0·097
	7/8	22·2	22·5	0·090	30·8	31·6	0·62	18·2	0·134	11·0	0·083	14·7	0·092
	6/8	19·1	19·5	0·095	28·4	29·2	0·63	17·8	0·148	11·2	6 111	14·6	0·097
	5/8	15·9	15·5	0·102	30·8	31·0	0·60	18·9	0·157	12·0	0.116	15.6	0·125

* Standard error based on 200 readings

II

of results

In μ						Immaturity (per cent)					
Max. / Min.	Fibre diameter	S. E.	Wall-thickness	S. E.	Col. 16 / Col. 18	Ripe	S. E.	Dead	S. E.	Thin-walled	S. E.
15	16	17	18	19	20	21	22	23	24	25	26
2·59	22·8	0·106	7·5		3·64	46	2·5	41	2·4	13	1·7
2·61	23·2	0·123	7·3		3·78	41	2·4	41	2·4	18	1·9
2·69	23·8	0·138	8·6		3·48	55	2·5	29	2·2	16	1·8
2·37	24·0	0·132	9·4		3·16	65	2·4	22	2·0	13	1·7
2·62	24·4	0·174	9·2		3·30	62	2·4	26	2·2	12	1·6
2·90	24·0	0·198	8·8		3·44	58	2·4	30	2·0	12	1·6
2·82	20·6	0·110	7·3		3·52	52	2·5	34	2·4	14	1·8
3·20	22·2	0·110	7·0		3·84	48	2·5	44	2·4	8	1·4
3·08	23·0	0·123	8·1		3·36	60	2·4	29	2·2	11	1·6
2·84	24·0	0·136	9·4		3·18	68	2·4	20	2·0	12	1·6
3·16	24·4	0·176	9·0		3·42	62	2·4	27	2·2	11	1·6
3·97	24·1	0·196	8·0		4·02	50	2·5	38	2·4	12	1·6
2·13	21·8	0·100	8·5		2·76	75	2·2	8	1·4	17	1·9
2·16	23·0	0·106	8·8		3·04	72	2·2	15	1·8	13	1·7
2·59	23·8	0·117	8·5		3·60	66	2·4	28	2·2	6	1·2
3·16	24·5	0·132	8·4		4·14	56	2·4	38	2·4	6	1·2
3·72	24·8	0·132	7·9		4·42	52	2·5	44	2·4	4	1·0
1·640	21·8	0·172*	10·7	0·089	2·058	99·5	0·5	0·0	0·0	0·5	0·5*
1·639	22·6	0·162	11·1	0·096	2·050	99·0	0·7	0·5	0·5	0·5	0·5
1·623	23·5	0·172	11·5	0·104	2·060	98·5	0·8	0·5	0·5	1·0	0·7
1·644	24·1	0·183	11·8	0·121	2·044	98·5	0·8	1·5	0·8	0·0	0·0
1·574	25·8	0·218	12·8	0·123	2·032	99·0	0·7	0·5	0·5	0·5	0·5

only for this cotton ; for the other three cottons on 400 readings.

It will be seen that in both the irrigated and rain-fed samples of Co 2, the ripe fibres decrease slightly from 10/8 in. to 9/8 in. after which they increase up to 7/8 in. and then again decrease (Table II, Col. 21 ; Fig. 2). The dead fibres, as should be expected, record an opposite tendency, there being a fall from 9/8 to 7/8 followed by a rise. Though the fall of maturity from the 10/8 in. group-length to the 9/8 in. is not significant, its persistance in both the samples of Co 2 and in the other tests described below should indicate to its real existence. In Karunganni the variation is in a single direction, there being a fall in the ripe and thin-walled fibres and a rise in the dead fibres with the decrease in length. Almost all the differences in the case of the dead fibres and many in the case of the ripe fibres are statistically significant. In the strain 2405 there is negligible immaturity with little variation. It will thus be seen that there are distinct variations of maturity with respect to length in some strains, the trend of variation depending upon the strain of cotton.

To have a comparative idea of the results obtained according to Clegg's method with those derived according to the present method, the fibres in the different length-grades were also examined by Clegg's method by testing five bunches of about hundred fibres each for each length. The results obtained are given in Table III.

On comparison with Table II, columns 21, 23 and 25 it will be seen that in most of the cases the differences between the values obtained according to the two methods are well within sampling errors. The trends of variation with respect to length, that can be deduced from these results are practically identical with those according to the other method (Figs. 2 and 3). It is remarkable to note that the very simple method adopted by Clegg gives results which are nearly as reliable as those got by the method which is very much more elaborate and time-absorbing.

With regard to the variation of the maturity in the different lengths, Underwood [1930] states that " As a general rule the short hairs contain higher proportions of immature cotton than the longer hairs." This can be conceived in a bulk sample badly ginned by machinery where many of the long immature fibres may break and thus increase the amount of immature cotton in the shorter lengths. In the present case the increase of the immature fibres in the shorter lengths in the strain Co 2, especially after a decrease up to the 7/8 in. length, may lead one to suspect that it may be due to a similar cause, in view of the fact that the proportion of the immature fibres in the longest length is very great in this cotton. But since great care was taken against any breakage during the preparatory processes the influence of this factor should not be considerable.

TABLE III

Immaturity percentage according to Clegg's method

	Group length in 1/8 in.	Co 2 irrigated		Co 2 rain-fed		Karunganni	
		Value	S. E.	Value	S. E.	Value	S. E.
Ripe	10	50	2·2	51	2·2	..	..
	9	49	2·2	45	2·2	82	1·7
	8	61	2·2	62	2·2	74	2·0
	7	62	2·2	62	2·2	59	2·2
	6	58	2·2	56	2·2	50	2·2
	5	47	2·2	52	2·2	46	2·2
Dead	10	32	2·1	31	2·1	..	..
	9	33	2·1	38	2·2	9	1·3
	8	24	1·9	25	1·9	17	1·7
	7	27	2·0	24	1·9	30	2·1
	6	30	2·1	27	2·0	37	2·1
	5	42	2·2	33	2·1	41	2·2
Thin-walled	10	18	1·7	18	1·7	..	..
	9	18	1·7	17	1·7	9	1·3
	8	15	1·7	13	1·5	9	1·3
	7	11	1·4	14	1·6	11	1·4
	6	12	1·4	17	1·7	13	1·5
	5	11	1·4	15	1·6	13	1·5

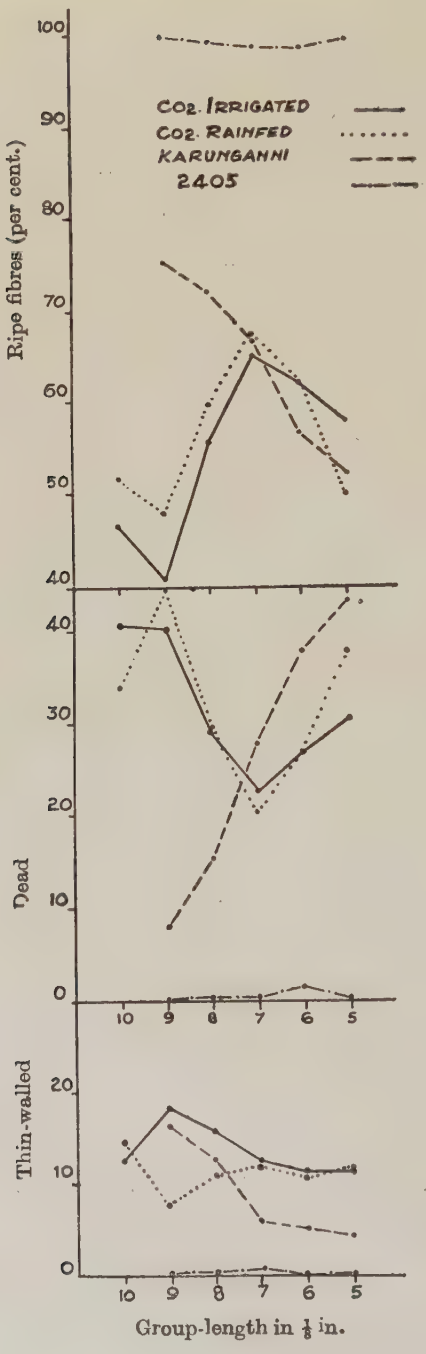

Co2. IRRIGATED
Co2. RAINFED
KARUNGANNI
2405
Ripe fibres (per cent.)
Dead
Thin-walled
Group-length in 1/8 in.

FIG. 2

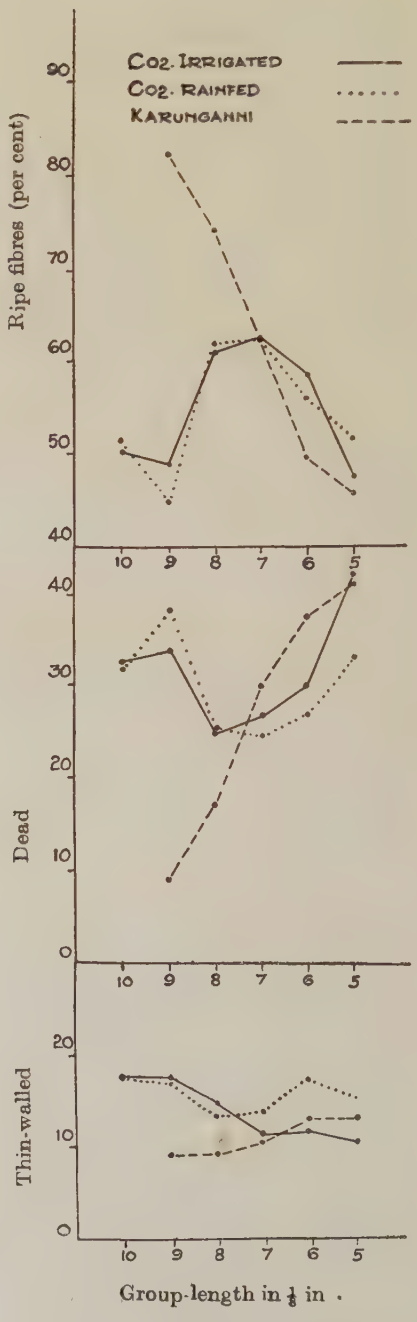

Co2. IRRIGATED
Co2. RAINFED
KARUNGANNI
Ripe fibres (per cent)
Dead
Thin-walled
Group-length in 1/8 in .

FIG. 3

(*b*) *Convolutions*

The frequency distribution of this character is recorded in Appendix I. It will be seen that the distribution is considerably skew excepting in the strain 2405 where it is normal. In the first three cottons the frequency in the first class-interval is so high that it makes the frequency polygon look like a combination of a normal curve with an exponential curve (Fig. 4 *a*). If the ripe fibres alone are considered, the distribution is normal (Appendix II).

The mean values of the number of convolutions per centimeter are given in Table II, column 6. As the number of fibres tested is considerably less than required for the correct estimation of this property, and as the observed distribution is very skew in all samples but one, the conclusions drawn should be considered to be only very approximate.

In the irrigated sample of Co 2 the number of convolutions per centimeter increase from 25·4 for the longest group-length to 50·6 for the 7/8 in. with a fall to 40·6 for the 5/8 in. In the same strain grown under rain-fed conditions the variation is not so large. It can be said to be constant in all the group-lengths at about 45 except for a rise to 59·8 in the 7/8 in. which group-length, it is interesting to note, records the highest value in the irrigated sample also. In Karunganni on the other hand the variation is more distinct and is only in one direction. It is 38·8 for the longest length and 20·0 for the shortest, the fall appearing to be gradual in the intermediate lengths. In the strain 2405 no regular trend of variation is apparent though some differences are significant.

The relationship between the convolutions and the maturity of the fibre may now be considered employing the ratio of fibre-diameter to wall-thickness as a measure of the maturity. The correlation coefficient cannot be calculated by the product-moment method as both the characters are not normally distributed. The modifications suggested by Yule [1929], namely, plotting the mean value of one of the characters corresponding to each group-interval of the other has been employed as shown in Fig. 5.

It will be seen that the regression lines are not linear, but appear to be portions of a rectangular hyperbola. The number of convolutions decreases with the decrease in the maturity. The ripe fibres (limits of the ratio 2·0 to 3·0) contain the largest number of convolutions, the thin-walled fibres (3·0 to 4·0) contain a lesser number and the dead fibres (above 4·0) the least.

It therefore follows that in the convolutions frequency-distribution curve (Fig. 4*a*) the portion corresponding to the small number of convolutions belongs to the immature fibres and that to the larger number to the ripe fibres ; that is, the ' exponential ' part of the curve belongs to the immature and the ' normal ' part to the ripe fibres. This finding is in agreement with the statements of Denham [1923], Clegg [1932] and Gulati and Ahmad [1935].

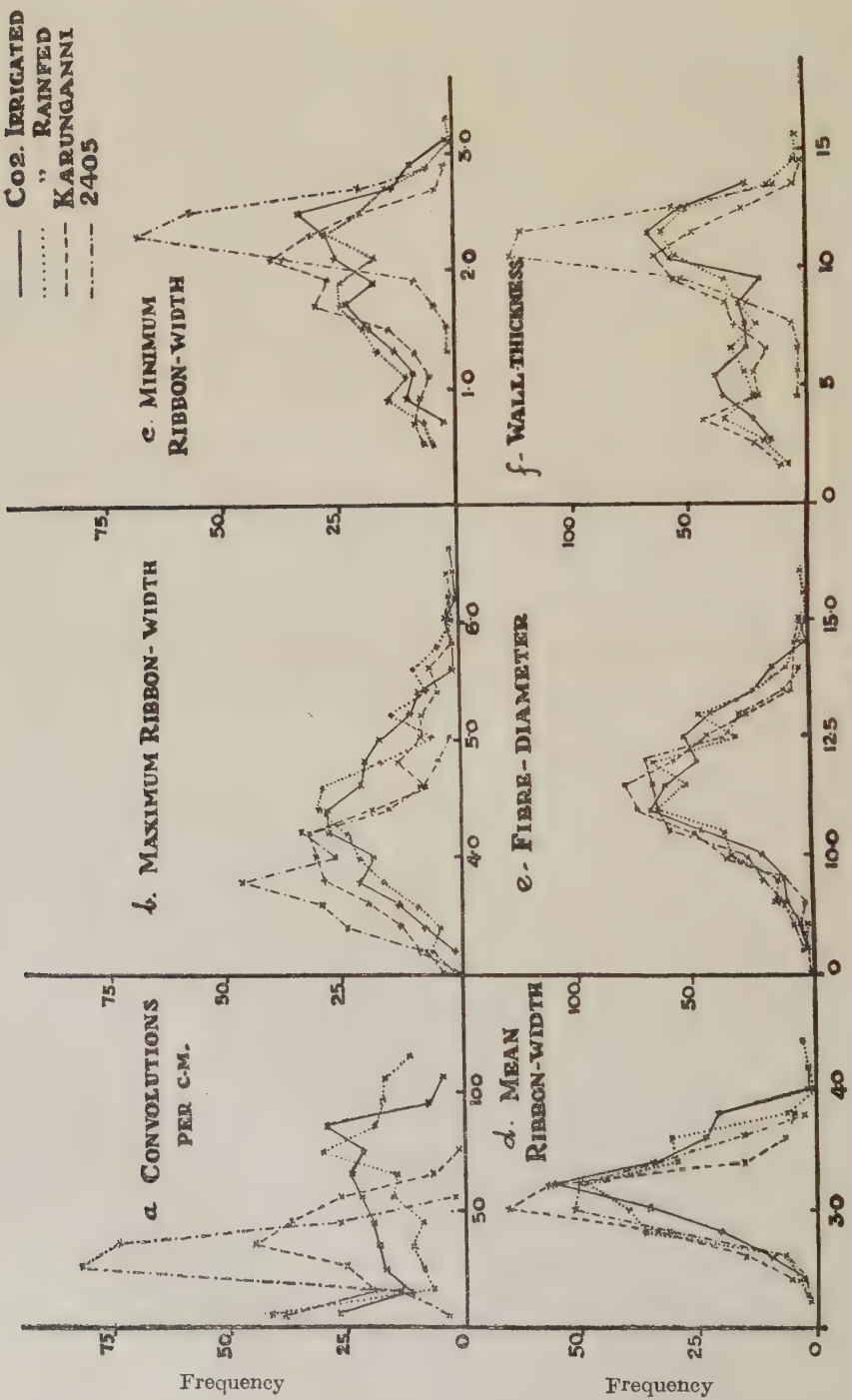

Co2. Irrigated
" Rainfed
Karunganni
2405
a Convolutions per c.m.
b. Maximum Ribbon-width
c. Minimum Ribbon-width
d. Mean Ribbon-width
e- Fibre-diameter
f- Wall thickness
Frequency
Frequency

FIG. 4

CO2. IRRIGATED
CO2. RAINFED
LONGEST
MEDIUM 7/8"
KARUNGANNI
MEANS OF CONVOLUTIONS
MEAN OF RATIO
Ratio of fibre-diameter to wall-thickness
Number of convolutions per centimeter

FIG. 5

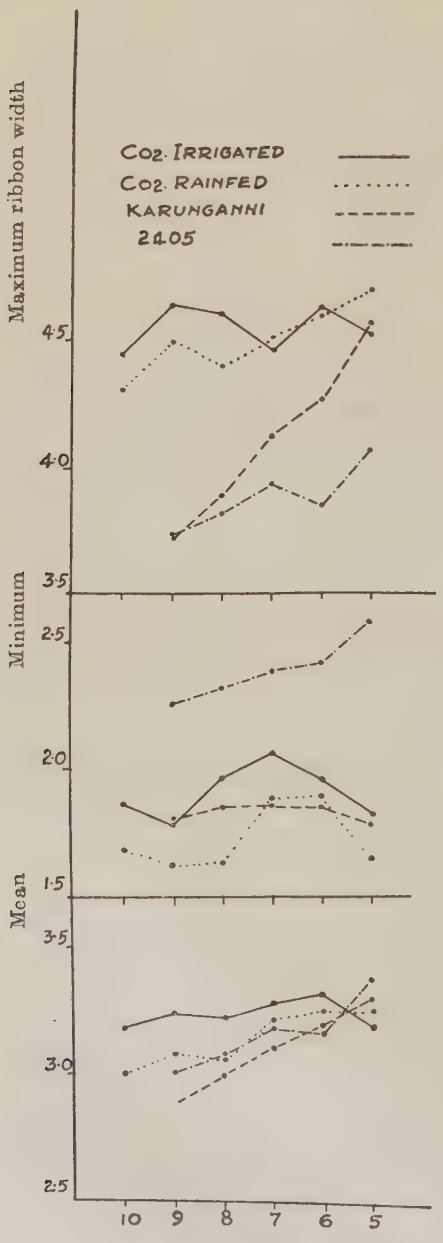


FIG. 6

(c) *Maximum ribbon-width*

The frequency distribution of this character is given in fig. 4*b**. For practical purposes the distribution can be taken as normal, though in some cases where the immaturity is large there is a tendency for the curve to get tailed at the farther end. It is obvious that the collapsed immature fibre, exhibiting a large ribbon-width, is responsible for this tailing. The distribution obtained by Koshal and Turner [1930] for this property is also normal. The mean values are given in Table II, col. 9.

In the rain-fed sample of Co 2 the variation is regular, *viz.*, rising steadily with the decrease of length. In the same strain grown under irrigation, though some of the values are significantly different from the others the regularity of the variation is not so apparent. Comparing the mean values for the irrigated with the rain-fed sample, it will be seen that irrigation appears to increase the maximum ribbon-width in the longer lengths (Fig. 6). In Karunganni the variation is more pronounced. Most of the differences are large and all of them significant and the value increases rapidly with the decrease in length. In strain 2405 the trend of variation is similar (except for the 6/8-in. group-length) though of a lower degree.

* For want of space the frequency distributions for all the group-lengths are not given in the paper. The frequencies for the one inch group-length are presented in Fig. 4, that length being the modal group-length for three out of the four cottons studied.

(*d*) *Minimum ribbon-width*

The distribution appears to depart from normality in Co 2 and Karunganni ; the curve is much flattened and has often a tendency to become bi-modal in these strains as can be seen in Fig. 4*c*. This behaviour is again due to the presence of immature fibres. These fibres on account of their being thin, crowd about the smaller values and when the immaturity is large there is an inflation at this part of the curve, tending to make it depart from normality, and if the immaturity is sufficiently large this inflation makes the curve even bi-modal. All the group-lengths of Co 2 and the shorter ones of Karunganni record such distributions. The distribution tends to approach normality in the longer lengths of Karunganni probably on account of the small percentage of immature fibres in these lengths. In strain 2405, where there is practically negligible immaturity, the distribution is normal. The writer is not aware of any previous record of the distribution of this character.

The significances of the differences between the mean values can be assessed by the use of the standard error in strain 2405 only. The mean values are given in Table II, col. 11.

It will be seen that both in Co 2 and in Karunganni the differences are small and there is no apparent trend of variation. In strain 2405 the differences are all significant and the trend is distinct, *viz.*, increasing with the decrease of length (Fig. 6).

Irrigation appears to increase the value of the minimum ribbon-width especially in the longer lengths, as was found in the case of the maximum ribbon-width.

(*e*) *Mean ribbon-width*

This attribute is calculated along the lines described by Barritt [1930] as the mean of the maximum and minimum ribbon-widths. The distribution appears to be normal in all cases (Fig. 4*d*) and the standard error is very small when compared with that of the maximum ribbon-width. The immature fibres do not affect this property as they do the other two ribbon-widths. The mean values are recorded in Table II, col. 13.

In Co 2 irrigated some differences are statistically significant for odds 20 : 1 and there is a faint tendency for the mean ribbon-width to increase with the decrease of length (Fig. 6). In the rain-fed sample of the same strain the same tendency is more apparent. In Karunganni the trend of variation is the same and all the differences are significant. In strain 2405 also the behaviour is identical. Hence it can be stated that the general tendency is for the mean ribbon-width to rise steadily with the decrease of length.

The effect of irrigation appears to be to increase the mean ribbon-width, more especially in the longer lengths.

The values of the correlation coefficient between mean ribbon-width and fibre length, when all the fibres in all the group-lengths are taken together, has been worked out and these are given in Table IV.

TABLE IV

Cotton	Value of coefficient	N	Value for significance		Remarks
			$P=0\cdot5$	$P=0\cdot1$	
Co 2 irrigated . .	−0·066	1200	0·0577	0·0750	Significant
Co 2 rain-fed . .	−0·274	1200	0·0577	0·0750	Highly significant
Karunganni . .	−0·479	1000	0·0632	0·0822	Highly significant
Strain 2405 . .	−0·346	1000	0·0632	0·0822	Highly significant

It is seen that all the coefficients are negative and significant.

Taking the mean ribbon-width as a measure of the fineness of cotton, it can therefore be stated that the fineness improves with increase of length. According to Balls [1928] the " general tendency to correlation between length and fineness throughout genus *Gossypium* " is also the same. The association of fineness with length thus appears to be a general rule.

In discussing the usefulness of the mean ribbon-width Barritt [1930] states " the two extreme measurements cause considerable increase in the dispersion due to the immature hairs, so that the standard deviation of the mean becomes a useful measure of the degree of immaturity of the sample." The results obtained in the present investigation may incidentally be utilised to verify this conclusion. The results are recorded in Table V. The strain 2405 has been omitted as there is negligible amount of immaturity in this strain.

TABLE V

Group length in 1/8 in.	Co 2 irrigated			Co 2 rain-fed			Karunganni		
	S. E.	Ripe fibres (per cent)	Dead fibres (per cent)	S. E.	Ripe fibres (per cent)	Dead fibres (per cent)	S. E.	Ripe fibres (per cent)	Dead fibres (per cent)
10	0·020	46·5	40·8	0·020	51·5	34·0			
9	0·020	41·0	40·5	0·020	47·8	44·5	0·017	75·2	8·0
8	0·023	55·5	28·8	0·019	59·5	29·5	0·018	72·0	15·2
7	0·024	64·8	22·5	0·023	67·5	20·5	0·019	66·8	27·8
6	0·031	62·0	26·5	0·028	61·8	27·2	0·021	56·5	38·0
5	0·030	57·7	30·5	0·031	50·0	37·8	0·020	52·2	43·5

The results indicate that in both the samples of Co 2 the standard error increases steadily with the fall in length in spite of the significant variations of the immaturity in nearly the opposite direction. In Karunganni, though there is a large rise in the immaturity with the decrease in length, the variation of the standard error is very little. It is much smaller than that found even in the strain Co 2, where the variation in the maturity is less marked. Hence the conclusion of Barritt, namely, that standard error of the mean ribbon-width being a measure of the maturity, cannot be said to be borne out in the samples under study.

(*f*) *Swollen fibre-diameter*

The diameter measured in the present enquiry corresponds to the swollen diameter studied by Calvert and Summers [1925] and by Barritt [1929] with this difference, namely, that in the present case the fibre is held tight at the two ends so that it is not allowed to contract during the swelling process. The frequency distributions are given in Fig. 4*e*. It may be recalled that the number of fibres tested is 400 in the first three cottons and 200 in the fourth, namely, strain 2405. The mean values are recorded in Table II, col. 16.

In the strain Co 2, both irrigated and rain-fed, the fibre-diameter increases steadily with the decrease in length up to 6/8 in. with a slight fall in the 5/8 in. (Fig. 7). Most of the differences, even between consecutive group-lengths, are significant. In Karunganni also the diameter increases with the fall in length ; the difference between 6/8 in. and 5/8 in. alone is not, however, statistically significant. In strain 2405 also the same trend of variation is noticed and all the differences are significant. The increase of fibre-diameter with the decrease in length appears thus to be the general rule.

The effect of irrigation in the strain Co 2 appears to be to increase the diameter in the longer lengths, as was found to be the tendency in the case of the ribbon-widths, indicating that the fibre-cells tend to grow bigger under irrigation.

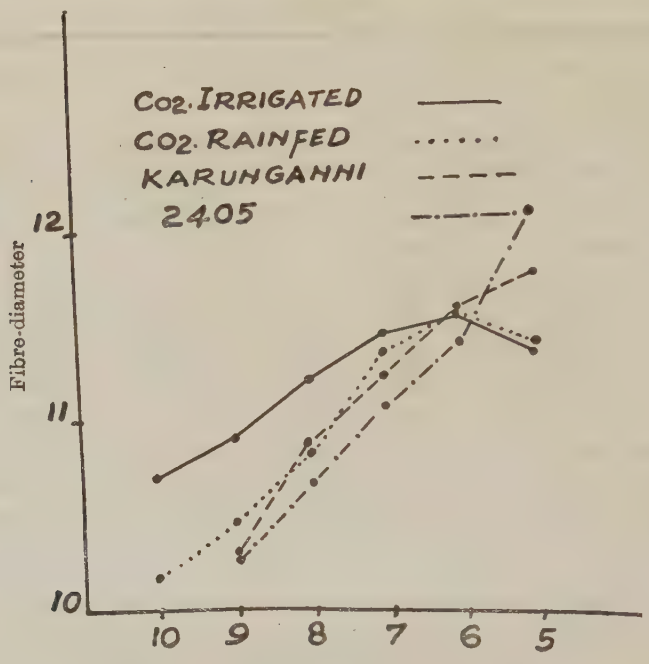


FIG. 7

(*g*) *Wall-thickness*

The distribution of this character (Fig. 4*f*) is similar to that of the minimum ribbon-width. The mean values are given in Table II, col. 18.

In the strain Co 2 and Karunganni the variations do not exhibit any definite trend (Fig. 8). In strain 2405, however, the wall-thickness increases steadily with the decrease in length and all the differences are significant.

(*h*) *Weight of dead and thin-walled fibres*

From the mean values of the ratio $D/D—d$ for the different categories of fibres, it is possible to determine the weights of the thin-walled and dead fibres in terms of the fully ripe fibre. The volume of cellulose in the fibre having a diameter, D, and internal canal of diameter, d, is equal to $\frac{\pi}{4}(D^2—d^2)$ per unit length. The volume of a fully ripe fibre of the same diameter is $\frac{\pi}{4} D^2$ for the same length. Assuming the density of the cellulose to be the same in both *, the ratio of the weights of the two is given by $\frac{D^2—d^2}{D^2}$

* This assumption may not be perfectly valid because the swollen cellulose is under pressure inside a ripe fibre while there is not so much pressure inside an immature fibre, there being more available space for expansion inside it. Due to the higher pressure the density may be a little more in the ripe fibre.

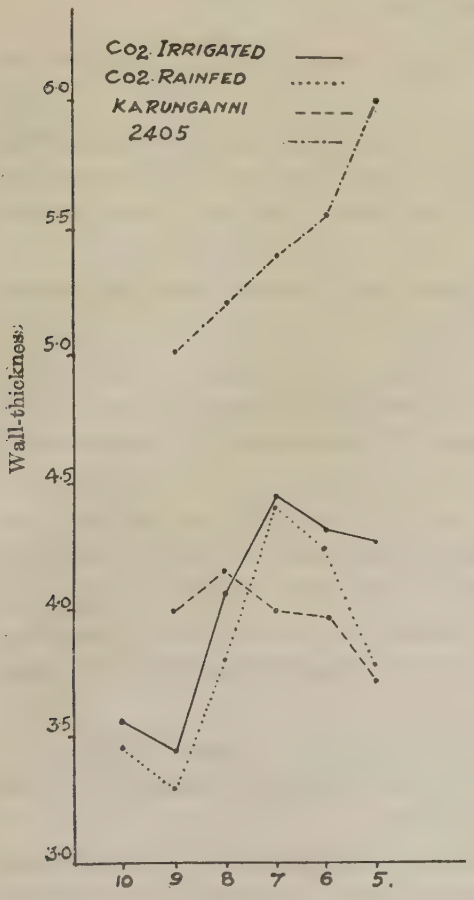


FIG. 8

The value of the ratio $D/D—d$ for the ripe fibres ranges from 1·0 to 1·5; most of the values, however, lie in the neighbourhood of 1·0 and the mean value comes to less than 1·1. Calculating from this value the weight of a fibre in the 'ripe' class comes to 0·992 of a perfectly ripe fibre, whose weight is taken as 1·0. For the thin-walled fibres the ratio ranges from 1·5 to 2·0 and the mean can be taken as 1·75. This gives the weight of the thin-walled fibre as 0·816 of the perfectly ripe. For the dead fibres the mean value of the ratio varies rather widely, ranging from 2·5 to 5·0 or even more in a few cases. But for most of them the mean can be taken as 3·3 which gives the weight as 0·487.

The values got by Peirce and Lord [1934] by actual weighing are 0·75 and 0·45 respectively for the thin-walled and dead fibres. The figures obtained by them from microscopical measurements also are nearly the same, being 0·69 and 0·74 for the thin-walled and 0·38 for the dead. The results obtained in the present case should, therefore, be considered to be a little higher. This might be due to the choice of the smaller upper limit (4 and not 5, as according to these authors) for the thin-walled fibres in the present case as already referred to on page 311.

V. Conclusions

The following conclusions can be drawn from the results considered in the foregoing :—

(1) The variation of the maturity of fibres with respect to length is different in different strains ; further work is to be done before it is possible to state that a particular variation is due to strain or culture.

(2) The convolutions per unit length also indicate different trends of variation in the different cottons.

(3) The maximum ribbon-width, mean ribbon-width and swollen diameter generally decrease with the increase of length, indicating the general tendency of improvement of fineness with length.

(4) The minimum ribbon-width and swollen wall-thickness are not reliable measures on account of their skew distributions in three out of four cottons studied in the present work.

(5) The number of convolutions per unit length has a definite relationship with the maturity of the fibre, decreasing with the decrease in the degree of maturity.

(6) A general survey of the standard errors discloses the fact that fibres are more uniform in their physical properties in the longer lengths than in the shorter.

(7) The effect of irrigation in strain Co 2 appears to be to increase the diameter of the fibre-cell, more especially in the longer lengths.

(8) The mean weights of the thin-walled and dead fibres, calculated from the respective mean values of the ratio of swollen fibre-diameter to swollen wall-thickness, are found to be slightly greater than the values obtained by Peirce and Lord.

VI. Acknowledgments

The writer wishes to record his best thanks to Rao Bahadur V. Ramanatha Ayyar for his valuable advice and sympathetic interest in the work. He is equally thankful to Dr. Nazir Ahmad for his considered criticisms and valuable suggestions. The work was carried out under the financial assistance of the Indian Central Cotton Committee, Bombay.

REFERENCES

Ahmad, N. and Gulati, A. N. (1936). *J. Text. Inst.* **27**, T109-T111

Balls, W. L. (1915). "Development and Properties of Raw Cotton": A. & C. Black, Ltd.

————(1928). "Studies of Quality in Cotton": Macmillan & Co.

Barritt, N. W. (1929). *Ann. App. Biol.* **16**, 438-443

————(1930). *Emp. Cotton Grow. Rev.* **7**, 19-29

Calvert, M. A. and Summers, F. (1925). *Shirley Inst. Mem.* **4**, 49-84

Clegg, G. G. (1932). *J. Text. Inst.* **23**, T35-T54

Denham, H. J. (1923). *Shirley Inst. Mem.* **2**, 61-88

Editor (1930). *Emp. Cotton Grow. Rev.* **7**, 261-274

Fisher, R. A. (1935). "Statistical Methods for Research Workers" (Fifth Edition): Oliver & Boyd

Gulati, A. N. and Ahmad, N. (1935). *Ind. Cent. Cot. Com. Tech. Lab., Tech. Bull.* (*Ser. B*), No. **20**

Iyengar, R. L. N. and Turner, A. J. (1930). *Ind. Cent. Cot. Com. Tech. Lab., Tech. Bull.* (*Ser. B.*), *No.* **7** (*J. Text. Inst.*, 1930, **21**, T417-T440)

Koshal, R. S. and Turner, A. J. (1930). *Ind. Cent. Cot. Com., Tech Lab., Tech. Bull.* (*Ser. B*), *No.* **6**; *No.* **10**

Peirce, F. T. and Lord, E. (1934). *Second Conference on Cotton Growing Problems, Report*, 1934, pp. 223-244

Pope, O. A. (1933). *J. Amer. Soc. Agron.* **25**, 740-756.

Turner, A. J. (1929). *Ind. Cent. Cot. Com., Tech. Lab., Tech. Bull.* (*Tech. Ser.*) *No.* **13**

Underwood, C. (1930). *Conference on Cotton Growing Problems, Report*, 1930, p. 14

Yule, G. U. (1929). "An Introduction to the Theory of Statistics": G. Griffin & Co. Ltd.

Appendix I

Convolutions per cm. (all fibres)

Class interval	Co 2 irrigated						Co 2 rain-fed						Karunganni					Class interval	Strain 2405				
	10/8	9/8	8/8	7/8	6/8	5/8	10/8	9/8	8/8	7/8	6/8	5/8	9/8	8/8	7/8	6/8	5/8		9/8	8/8	7/8	6/8	5/8
0—10 . . .	82	69	50	27	56	67	59	80	69	41	65	85	22	29	39	55	84	0—5 .	...	2	3	4	1
11—20 . . .	16	23	9	12	8	9	13	3	12	6	13	7	22	18	19	19	20	6—10 .	3	0	0	2	1
21—30 . . .	12	17	14	17	7	12	6	5	7	8	6	10	24	39	25	28	32	11—15 .	9	4	3	4	5
31—40 . . .	26	20	11	18	11	8	9	10	7	11	10	9	33	33	45	50	34	16—20 .	29	7	10	19	17
41—50 . . .	28	21	23	19	19	13	13	9	11	8	13	9	33	38	37	35	27	21—25 .	46	28	30	34	32
51—60 . . .	23	14	29	22	12	18	23	15	8	15	12	15	34	32	26	10	3	26—30 .	58	37	52	50	50
61—70 . . .	9	14	24	24	19	12	26	13	17	14	23	15	22	10	6	3	...	31—35 .	37	47	41	51	46
71—80 . . .	4	13	19	21	30	23	19	18	18	30	23	17	8	1	1	...	...	36—40 .	17	48	33	19	26
81—90 . . .	...	7	12	29	25	16	20	12	17	19	19	12	1	...	...	...	...	41—45 .	1	21	16	13	16
91—100 . . .	...	2	7	7	6	7	7	15	17	17	8	8	1	...	...	...	...	46—50 .	...	4	10	3	3
101—110 . . .	...	...	2	4	5	3	2	10	4	17	5	6	...	...	...	...	...	51—55 .	...	2	2	0	3
111—120 . . .	...	...	...	...	2	1	3	8	6	11	2	2	...	...	...	...	...	56—60 .	...	...	...	0	...
121—130 . . .	...	...	...	...	...	1	...	2	6	0	1	3	...	...	...	...	...	61—65 .	...	...	...	0	...
131—140 . . .	...	...	...	...	...	...	...	...	0	1	...	2	...	...	...	...	...	66—70 .	...	...	...	0	...
141—150 . . .	...	...	...	...	...	...	...	...	1	2	...	...	...	...	...	...	...	71—75 .	...	...	...	1	...

APPENDIX II

Convolutions per cm. (ripe fibres only)

Class interval	Co 2 irrigated						Co 2 rain-fed						Karunganni					Class interval	Strain 2405				
	10/8	9/8	8/8	7/8	6/8	5/8	10/8	9/8	8/8	7/8	6/8	5/8	9/8	8/8	7/8	6/8	5/8		9/8	8/8	7/8	6/8	5/8
0—10	1	...	...	1	1	2	...	...	1	2	1	1	...	1	1	...	3	0—5	...	...	...	1	...
11—20	6	...	1	3	3	2	...	1	0	0	3	2	9	10	15	12	18	6—10	2	...	...	2	1
21—30	11	4	1	3	4	3	...	2	1	1	3	5	14	28	19	26	32	11—15	9	4	3	4	5
31—40	25	10	5	14	6	7	2	10	2	6	9	6	29	31	46	50	34	16—20	29	7	10	19	17
41—50	28	20	18	18	14	13	5	7	7	6	13	7	33	38	37	35	27	21—25	46	28	30	34	32
51—60	23	13	27	21	11	18	20	15	8	14	11	12	34	32	26	10	3	26—30	58	37	52	50	50
61—70	9	14	23	23	19	21	25	13	17	14	23	15	22	10	6	3	...	31—35	37	47	41	51	46
71—80	4	13	19	21	30	23	19	18	18	29	24	17	8	1	1	...	...	36—40	17	48	3	19	26
81—90	...	7	12	29	25	16	19	12	16	18	19	12	1	...	...	...	...	41—45	1	21	16	13	16
91—100	...	2	7	7	6	7	7	15	17	17	8	8	1	...	...	...	...	46—50	...	4	10	3	3
101—110	...	...	2	4	5	3	2	10	4	17	5	6	...	...	...	...	...	51—55	...	2	2	0	3
111—120	...	...	...	...	2	1	3	8	6	11	2	2	...	...	...	...	...	56—60	...	...	...	0	...
121—130	...	...	...	...	...	1	...	2	6	0	1	3	...	...	...	...	...	61—65	...	...	...	0	...
131—140	...	...	...	...	...	...	...	...	0	1	...	2	...	...	...	...	...	66—70	...	...	...	0	...
141—150	...	...	...	...	...	...	...	...	1	2	...	...	...	...	...	...	...	71—75	...	...	...	1	...

STUDIES ON THE ROOT-ROT DISEASE OF COTTON IN THE PUNJAB

VI. CHEMICAL COMPOSITION OF HEALTHY AND DISEASED COTTON PLANTS

BY

R. SAHAI VASUDEVA, B.Sc., Ph. D. (Lond.), D.I.C. (Lond.)

Assistant Cotton Mycologist

AND

MOHAMMAD RAFIQUE, M.Sc.

Agricultural Assistant, Department of Botany, Agricultural Research Institute, Lyallpur

(Received for publication on 15th July 1938)

CONTENTS

		Page
I.	Introductory	331
II.	Material and method	332
	A. Sample for carbohydrate analysis	332
	B. Sample for chemical analysis	333
III.	Experimental	333
	A. Carbohydrates	333
	B. Nitrogen	334
	C. Chemical analysis	336
	(*a*) Estimation of ash, iron and phosphorus	336
	(*b*) Estimation of potassium, sodium and calcium	338
	(*c*) Total sugars and potassium ratio	340
	(*d*) Distribution of various elements in the leaf and root	341
IV.	Summary	342
	References	342

I. Introductory

The present investigation is part of an extensive work which is being carried out in this Laboratory on the pathological aspect of the root-rot disease of cotton in order to discover control measures for this serious disease. The work includes quantitative study of changes that occur in the plants that have died as a result of the root-rot disease.

Plants that have recently wilted on account of the attack of root-rot have all their root tissues, particularly the bark, broken down into shreds and can be easily pulled out of the ground. The disease actually starts much earlier and the manifestation of the disease above ground in the form of wilt is a very late symptom. Roots of diseased plants are moist and sticky to the touch, and leave a yellow stain on white surface [Vasudeva, 1935].

The disease invariably appears in patches, but sometimes when two plants are growing at the same spot only one of them might succumb to root-rot. The work reported in the present paper was undertaken with a view to determine the differences in the chemical composition of the diseased and healthy cotton plants growing in the same field.

II. Material and method

Sampling was carried out in plot No. 46/24 of the Botanical area at the Agricultural Research Station, Lyallpur. Samples of healthy and diseased cotton plants of variety 15-Mollisoni standing side by side in the same field were collected for analysis. Plants which had just begun to show the first signs of wilting, *i.e.*, the wilting of the topmost leaves, represented the diseased samples. As far as possible care was taken to select plants of the same age, size and height. The plants were dug out very carefully so that the greater part of the root-system was left intact. The digging, in order to expose the lowermost portion of roots, had to be carried out up to a depth of four feet. As soon as the entire plant was pulled out, it was divided into root, stem and leaf portions and each part was immediately put into separate air-tight glass jars. About thirty to fifty plants of each sort, *i.e.*, diseased and healthy, were worked into a composite sample which formed the final representative sample. Sampling was carried out throughout the season between 8 and 10 A.M. As soon as the samples were taken, they were carefully washed in a gentle spray of running water in order to remove the adhering soil particles. Care was always taken to prevent leaching during the cleaning operation.

The washed samples were carefully dried between folds of filter paper.

A. Sample for carbohydrate analysis

A portion of the fresh sample of root, stem and leaf of diseased and healthy plants was weighed and put into 90 per cent boiling alcohol. After a few minutes' boiling the samples were kept in air-tight jars with a few drops of toluene for further use.

The remaining material was put in hot air oven at 95°C. where it was left for about half an hour in order to stop the enzymic activity. The temperature of the oven was then brought down to about 50 to 55°C. The sample was left to dry at this temperature till constant weight was obtained. After the sample had dried, it was ground down to pass a 100-mesh sieve. The sample thus prepared was preserved in air-tight bottles and this formed the starting material for all subsequent analyses.

B. Sample for chemical analysis

The dried material was ashed in 500 c.c. porcelain dishes over the open Bunsen burner flame. The material was kept stirring with a glass rod in order to prevent fusion of the ash ingredients. The temperature of the ash under the conditions was not allowed to rise above 500°C. When almost constant weight of ash was obtained, it was preserved in air-tight bottles for further use. For the chemical analysis, a known weight of the ash was extracted with concentrated hydrochloric acid for eight hours as detailed in the Official Method [1936].

The extract thus prepared was evaporated to complete dryness over water-bath, where it was left for four to five hours to dehydrate silica. After dehydration of silica, the residue was taken up with dilute hydrochloric acid, made up to a known volume and filtered. The residue on the filter paper was washed, dried and ignited for the determination of the soluble silica content of the ash in the filtrate. The various mineral constituents were determined by methods described elsewhere.

III. Experimental.

A. Carbohydrates

The carbohydrates in the preserved samples were extracted with 80 per cent boiling alcohol using Soxhlet's extraction apparatus. The extraction was always allowed to proceed overnight. The extract was evaporated almost to dryness at 40°C. and the residue was taken up with distilled water. The gummy matter in the water extract was precipitated with lead acetate and the excess of lead acetate was removed by adding to the filtrate an extra quantity of sodium phosphate and filtering again. A few drops of toluene were added to the filtrate as a preservative. Reducing sugars and total sugars were estimated separately, the difference being reckoned as sucrose. The method followed for the determination of reducing and total sugars was essentially the same as described by Hanes [1929] and later by Hulme and Narain [1931]. The method consists in oxidising the reducing substances with excess of potassium ferricyanide. The excess of the reagent is made to liberate an equivalent amount of iodine from potassium iodide, which is titrated with standard thiosulphate solution.

The results are calculated on the basis of the fresh weight of the material taken. The results of the analyses are set out in Table I.

TABLE I

Distribution of carbohydrates in healthy and diseased cotton plants (mg. in 100 grams of fresh material)

Date of sampling	Material	Reducing sugars			Total sugars			Sucrose		
		H	D	*t*	H	D	*t*	H	D	*t*
19th June 1937	Root	303·0	674·0	10·9	1918·0	2725·0	2·5	1615·0	2051·0	3·4
29th June 1937		219·5	669·0		2106·0	3328·0		1986·5	2659·0	
13th July 1937		239·0	297·4		1168·0	1274·0		929·0	976·6	
27th July 1937		154·0	340·0		778·3	742·6		624·3	402·6	
13th Aug. 1937		113·3	191·0		495·4	1044·0		382·1	853·0	
19th June 1937	Stem	478·0	761·0	4·5	1345·0	2230·0	5·8	867·0	1469·0	5·7
29th June 1937		587·6	1130·6		1417·6	2690·0		829·4	1559·4	
13th July 1937		313·3	510·0		814·0	1505·0		500·7	995·0	
27th July 1937		285·0	424·0		778·3	...		493·3	...	
13th Aug. 1937		302·5	748·0		1009·0	2530·0		706·5	1782·0	
19th June 1937	Leaf	306·2	506·2	6·1	1239·0	1629·0	4·7	932·8	1122·8	2·8
29th June 1937		318·6	729·0		1060·0	1770·0		741·4	1041·0	
13th July 1937		283·2	789·1		920·1	1911·0		636·9	1121·9	
27th July 1937		347·0	644·0		566·3	955·6		219·3	311·6	
13th Aug. 1937		447·0	708·0		1115·0	2265·0		668·0	1557·0	

H = Healthy, D = Diseased.

The statistical significance of the differences in healthy and diseased plants was evaluated by ' Student's Method ' as described by Fisher [1925]. In this method a function, *t*, is calculated from the data and the probability of the result recorded being significant is greater or less according as this number is large or small. For a series of about five observations the value of ' *t* ' = 2·57 is on the margin of significance (the usual 20 : 1 standard being adopted).

The value of ' *t* ' for comparison of reducing sugars is fairly high, showing thereby that the results recorded are significant and that the reducing sugars in all parts of diseased plants, *i.e.*, root, stem and leaf are higher than in the respective parts of the healthy plants. The total sugars are significantly high both in the stem and leaf of the diseased plants. In the case of diseased roots the total sugars are high in four samples, but the results are negative in one sample.

Sucrose is also higher in the root, stem and leaf of the diseased plants.

B. NITROGEN

Nitrogen in the diseased and healthy plants was estimated as total nitrogen, nitrate nitrogen and ammoniacal nitrogen.

Total nitrogen was determined by the Official Gunning method [1936] modified to include the nitrogen of nitrates.

The inorganic fraction of nitrogen in the plant material was determined by the method described by Session and Shive [1928].

The method followed consists in distilling over the ammonia formed with a strong current of air. The free ammoniacal nitrogen in the plant material is first liberated with saturated sodium carbonate solution containing dissolved sodium chloride. The ammonia liberated is distilled over into standard acid contained in wide-mouthed glass tubes connected with the reaction flasks. A strong current of air is allowed to pass overnight both through the reaction flasks and the receivers. The system is disconnected and the excess acid is titrated against standard alkali. To the reaction flask is now added a known weight of Devarda's alloy and enough sodium hydroxide to give approximately $N/8$ solution in the reaction flask, from which acid is poured into the receiver tubes and a few drops of methyl red indicator added. Aeration is again allowed to proceed overnight and the excess acid is titrated against standard alkali. In this method there is no likelihood of any other portion of nitrogen, except the nitrate, coming over, as the entire experiment is conducted in the cold.

The results of the nitrogen estimations in different parts of healthy and diseased plants are given in Table II.

TABLE II

Distribution of nitrogen in healthy and diseased cotton plants (mg. per 100 gram dry matter)

Date of sampling	Material	NH_3—nitrogen			NO_3—nitrogen			Total nitrogen		
		H	D	t	H	D	t	H	D	t
19th June 1937	Root	10·0	19·0	2·9	214·0	186·0	5·2	1270·0	1630·0	11·8
29th June 1937		4·6	7·4		163·0	150·0		970·0	1390·0	
13th July 1937		...	...		...	...		1610·0	2070·0	
27th July 1937		3·2	6·5		170·0	136·0		1110·0	1380·0	
13th Aug. 1937		6·0	8·0		83·0	63·0		1180·0	1580·0	
19th June 1937	Stem	14·0	17·0	3·9	672·0	479·0	4·8	2210·0	2070·0	0·4
29th June 1937		14·0	20·0		409·0	357·0		1790·0	1820·0	
13th July 1937		4·2	13·5		425·0	234·0		2230·0	1940·0	
27th July 1937		8·3	17·7		414·0	289·0		1800·0	2000·0	
13th Aug. 1937		7·9	9·7		157·0	123·0		...	...	
19th June 1937	Leaf	42·0	48·0	3·4	108·0	134·0	2·7	4700·0	4440·0	1·5
29th June 1937		37·0	39·0		117·0	144·0		3820·0	3990·0	
13th July 1937		52·0	63·0		139·0	162·0		4570·0	4210·0	
27th July 1937		42·0	50·0		151·0	169·0		4220·0	4260·0	
13th Aug. 1937		29·0	31·0		111·0	103·0		4960·0	3520·0	

The following are the chief points of interest :—

(*a*) Total nitrogen in the diseased roots is significantly higher than in healthy roots.

(*b*) Ammoniacal nitrogen is significantly higher in roots, stems and leaves of the diseased plants.

(*c*) Nitrate nitrogen is significantly higher in roots and stems of healthy plants.

C. Chemical analysis

(*a*) *Estimation of ash, iron and phosphorus*

Ash.—This was determined according to the method already described.

Iron.—In samples of the hydrochloric acid extract of the plant material iron was estimated colorimetrically by Daniel and Harper's micro-method [1934]. This method consists in oxidising the iron into ferric state with a few drops of concentrated nitric acid and subsequently developing the colour with potassium sulphocyanide and comparing the tinge with controls containing known quantitities of iron. Standard iron solution was prepared by dissolving known weight of reagent grade ferrous ammonium sulphate in distilled water and oxidising with potassium permanganate. It was found during the course of these estimations that if either hydrochloric acid or nitric acid was omitted from the standard solution the shade of the colour developed with sodium sulphocyanide would not compare with that of the test solution. Accordingly the same quantities of hydrochloric acid and nitric acid were added to the control solution as were contained in the test solution.

Phosphorus.—Phosphorus was determined colorimetrically by the Official micro-method [1936]. The colour is developed with ammonium molybdate solution. Hydroquinine solution is then added to the test sample, the latter in the presence of sodium sulphite develops a blue colour with the solution containing phosphate. The tinge was compared with controls containing known amounts of phosphate.

The results of ash, iron and phosphorus estimations are given in Table III.

TABLE III

Per cent ash, iron and phosphorus in healthy and diseased cotton plants

Date of sampling	Material	Ash (grm. per 100 grm. dry matter)			Iron (mg. per 100 grm. dry matter)			Phosphorus (mg. per 100 grm. dry matter)		
		H	D	*t*	H	D	*t*	H	D	*t*
19th June 1937	Root	6·92	6·20	1·8	25·9	29·7	2·7	206·8	183·8	1·2
29th June 1937		5·33	5·84		17·3	23·9		45·9	79·6	
13th July 1937		8·71	6·64		38·2	40·6		121·4	112·8	
27th July 1937		5·75	5·07		21·3	28·6		143·1	127·0	
13th Aug. 1937		4·45	3·62		32·6	32·2		161·4	157·4	
19th June 1937	Stem	11·20	9·09	4.7	15·0	17·9	1·5	290·0	239·4	0·7
29th June 1937		8·62	7·42		11·5	10·6		111·0	123·0	
13th July 1937		9·42	6·98		19·3	16·0		126·5	114·1	
27th July 1937		8·30	6·59		21·7	16·1		152·5	142·0	
13th Aug. 1937		5·39	4·89		24·7	12·1		93·9	113·0	
19th June 1937	Leaf	20·40	17·70	2·0	43·1	27·9	1·8	378·0	360·0	0·2
29th June 1937		19·85	19·26		40·9	37·8		219·0	256·0	
13th July 1937		14·67	15·26		27·7	32·3		295·0	289·0	
27th July 1937		16·49	15·48		38·5	29·2		356·0	367·7	
13th Aug. 1937		16·82	13·96		33·0	24·6		252·0	238·0	

From the data the general conclusions deduced are as follows :—

(*i*) There is no significant difference in the ash of diseased and healthy roots and leaves.

Stems of diseased plants contain lower percentage of ash.

(*ii*) The diseased roots contain a higher amount of iron and the results are statistically significant.

The amount of iron in diseased samples of stems and leaves is lower in four out of five samples analysed, but the results are not significant.

(*iii*) There is no significant difference in phosphorus content of roots, stems and leaves of healthy and diseased plants.

(*b*) *Estimation of potassium, sodium and calcium*

Potassium.—Determinations of potassium were made according to the method described by Milne [1929]. Potassium was precipitated with cobaltinitrite reagent.

Sodium.—This was determined gravimetrically by precipitation with Kahn's reagent, *i.e.*, magnesium uranyl acetate [1930]. The precipitate was washed with alcohol and ether and weighed. Before the actual estimation of sodium and potassium, the test solutions were completely freed from extraneous elements. Throughout these estimations a reagent control was run side by side.

Calcium.—This was estimated in the hydrochloric acid extract microchemically by the Official method. Calcium was precipitated as oxalate at *p*H 5.

The results of potassium, sodium and calcium estimations are given in Table IV.

TABLE IV

Per cent potassium, sodium and calcium in healthy and diseased cotton plants (mg. per 100 grms. dry matter)

Date of sampling	Material	Calcium			Sodium			Potassium			Ca/K ratio		
		H	D	t	H	D	t	H	D	t	H	D	t
19th June 1937	Root	320·0	368·0		...	...		...	...		...		
29th June 1937		282·4	356·1		86·0	37·0		3644·0	2996·0		0·078	0·120	
13th July 1937		415·0	482·0	2·5	71·0	96·0	0·1	3677·0	2390·0	2·9	0·113	0·200	3·4
27th July 1937		327·0	334·0		44·0	65·0		1984·0	1818·0		0·165	0·184	
13th August 1937		351·0	349·0		40·0	35·0		1784·0	1203·0		0·197	0·290	
19th June 1937	Stem	1550·0	1250·0		86·0	85·0		1926·0	1420·0		0·805	0·880	
29th June 1937		1194·0	1108·0		77·3	53·0		2258·0	1910·0		0·529	0·580	
13th July 1937		2460·0	2410·0	2·3	69·0	41·0	3·0	3603·0	2350·0	1·4	0·683	1·020	0·0
27th July 1937		2533·0	1551·0		88·0	25·0		2631·0	1569·0		0·963	0·989	
13th August 1937		2155·0	1557·0		64·0	30·0		1801·0	2507·0		1·200	0·620	
19th June 1937	Leaf	5334·0	4440·0		88·0	79·0		1004·0	1021·0		5·310	4·350	
29th June 1937		5397·0	4845·0		112·0	114·0		3428·0	4307·0		1·570	1·120	
13th July 1937		3950·0	3690·0	5·1	75·0	104·0	0·3	2706·0	2672·0	1·1	1·460	1·380	2·4
27th July 1937		4170·0	3770·0		45·0	63·0		2108·0	2208·0		1·980	1·710	
13th August 1937		4210·0	3630·0		126·0	64·0		...	2606·0		...	...	

The data bring out the following points of interest.

(*i*) The roots of diseased plants contain less potassium.

(*ii*) The stems of diseased plants contain less sodium.

(*iii*) Diseased leaves contain significantly less calcium.

(*iv*) There are indications that the diseased roots contain a higher amount of calcium, results being on the margin of significance. The percentage of calcium in diseased plants as a whole is, however, significantly less.

(*v*) It is observed that calcium-potassium ratio is significantly higher in diseased roots.

(*c*) *Total sugars and potassium ratio*

The ratios deduced from the carbohydrate and potassium analyses are given in Table V.

TABLE V

Total sugars/potassium in healthy and diseased cotton plants

Sample	Material	Healthy	Diseased	*t*
1	Root	..	..	1·9
2		0·579	1·110	
3		0·318	0·533	
4		0·392	0·408	
5		0·278	0·868	
1	Stem	0·698	1·570	6·8
2		0·627	1·410	
3		0·226	0·640	
4		0·296	..	
5		0·560	1·010	
1	Leaf	1·230	1·600	4·4
2		0·309	0·411	
3		0·340	0·715	
4		0·269	0·433	
5		..	..	

It is clear from the data that total sugar/potassium is higher in stem and leaves of the diseased plants.

(*d*) *Distribution of various elements in the leaf and root*

Comparative distribution of iron, calcium and potassium in the leaf and root of healthy and diseased cotton plants is shown in Table VI.

TABLE VI

Comparative distribution of various elements in the leaf and root of healthy and diseased cotton plants

Sample	Fe in leaf/Fe in root			Ca in leaf/Ca in root			K in leaf/K in root		
	H	D	*t*	H	D	*t*	H	D	*t*
1	1·66	0·94		16·67	12·07		..	..	
2	2·36	1·58		19·11	13·61		0·94	1·44	
3	0·73	0·79	3·0	9·52	7·65	4·1	0·73	1·12	3·5
4	1·81	1·02		12·76	6.05		1·06	1·21	
5	1·01	0·76		12·0	10·4		..	2·17	

The results given above show that (*i*) the ratios of iron in leaf to iron in root and calcium in leaf to calcium in root are significantly lower in diseased plants. This means that although there appears to be accumulation of these two elements in the roots, the stems and leaves of the diseased plants are not able to make use of it. Further investigation must decide whether this condition is the result of disease or *vice versa*. (*ii*) the ratio of potassium in leaf to potassium in root is significantly higher in diseased plants.

Thanks are due to Professor J. C. Luthra, I.A.S., and Dr. P. E. Lander, I.A.S. for their keen interest throughout the course of this investigation. Thanks are also due to Dr. Ramji Narain for valuable suggestions and to the Indian Central Cotton Committee for financing the scheme.

The junior author carried out some of the analytical work under the guidance of the senior author.

IV. Summary

The diseased cotton plants which have wilted as a result of the root-rot attack exhibit marked differences in chemical composition as follows :—

1. Reducing sugars and sucrose are higher in all parts, *i.e.*, root, stem and leaf, of diseased than in the corresponding parts of healthy plants.
2. Total and ammoniacal nitrogen, iron and the ratio of calcium to potassium are significantly higher in diseased than in healthy roots.
3. The ratios of iron in leaf to iron in root and calcium in leaf to calcium in root are lower in diseased plants and the ratio of potassium in leaf to potassium in root is higher in diseased than in healthy plants.

REFERENCES

Daniel, H. A. and Harper, H. J. (1934). *J. Assoc. Official Agric. Chem. Wash.* **17,** 286-290

Fisher, R. A. (1925). "Statistical Methods for Research Workers." Edinburgh Oliver and Boyd

Haines, C. S. (1929). *Biochem. J.*, **23,** 99-106

Hulme, A. C. and Ramji Narain (1931). *Biochem. J.* **25,** 1051-61

Kahne, E. (1930). *Bull. Soc. Chim.* **47,** 382-402

Milne, G. (1929). *J. Agric. Sci.* **19,** 541-552

Session, A. C. and Shive, J. W. (1928). *Plant Physiol.* **3,** 500-511

Skinner, H. H. *et al.* (1935). 'Methods of Analysis of the Association of Official Agricultural Chemists' (4th Ed.) : Washington

Vasudeva, R. Sahai (1935). *Ind. J. Agric. Sci.* **5,** 496-512

NOTE

DECREE No. 24000/1936 F. M. OF ROYAL HUNGARIAN MINISTRY OF AGRICULTURE ON THE SUBJECT OF GOVERNMENT PHYTOSANITARY SERVICE REGULATIONS

Section 1

By reason of Articles 1, 2 and 3 of Law XVII of 1936 on the subject of the insertion of the International Agreement of Rome of April 16th, 1929, on plant protection, and based on Section 1, para. 2 of Decree No. 6300/1936 M. E. concerning the cessation of the Ministry in the Trade of Medicinal and Industrial Plants, I have decided on the constitution of a Government Phytosanitary Service to prevent and diminish loss caused to Farm and garden produce, whether by harmful insects, parasitic plants, or biological change.

Section 2

The objects of the Government Phytosanitary Service are as under : Co-operation with civil authorities in the enforcement of decrees and laws already passed or any future laws on the subject of the prevention of, and struggle against, loss in Farm and garden produce caused either by harmful insects, parasitic plants, or biological change. Among these laws and decrees we quote ; Art. XV of 1882 with reference to the insertion of the International Agreement of Berne of November 3rd, 1881, on the protective measures to be taken against " phylloxera vastatrix " as well as the complementary article of the law already mentioned. Art. XLI of 1889 ; Art. XVII of 1883 on the same subject ; Art. XII of 1894 on Agriculture and Supervision of Fields ; Executive clause No. 48,000 of 1894 of the preceding law ; Art. XXXI of 1907 on the destruction of the Moroccan Locust and Executive Decree No. 28,000 of 1907 of the preceding article ; Art. XLIV of 1925 on the precautions to be taken to prevent the arrival from abroad and spreading of potato diseases such as potato gangrene, and the Colorado Insect ; Executive Clause No. 40,000 of 1926 of the preceding Article ; Art. XVII of the 1936 law concerning the insertion of the international agreement of Rome of April 16th, 1929, on the protection of plants, the encouragement of administrative authorities to activity in that respect, and assistance by practical advice to producers concerned in their work of protection.

Section 3

Control of the Government Phytosanitary Service

The Government Phytosanitary Service is under the general control of the Royal Hungarian Ministry of Agriculture. [The actual department of the ministry that deals with this subject is the Phytosanitary Section IX. 4.]

Section 4

Departments of the Government Phytosanitary Service.

1. Consultative Body.
   Phytosanitary Council.
2. Experimental Body.
   Royal Hungarian Phytosanitary Institute.
3. Administrative Bodies.
   (*a*) Royal Hungarian Phytosanitary Service.
   (*b*) Royal Hungarian Phytosanitary Districts.
   (*c*) Local Phytosanitary Agents.

Section 5

The Phytosanitary Council is required to make recommendations to the Royal Hungarian Minister of Agriculture on any special question asked by this latter, and should make its proposals for its decisions.

The organization and Regulations of the Phytosanitary Council will be established by a special decree of the Royal Hungarian Minister of Agriculture.

Section 6

The Royal Hungarian Phytosanitary Institute, as the central body, has the following tasks :—

Theoretical and practical study of insects and parasitic plants, fungi and germs harmful to agricultural and garden produce, damage caused by biological change, as well as conditions of life of parasites and weeds.

Improvement on methods of protection, examination of preventative measures and protective machinery, laboratory examination and practical experiments, on different protective substances.

Organization on the ground of means of destruction of insects or parasitic plants which have made serious inroads in certain districts, by enlisting public participation or by asking for help from the State.

Practical advice and gratuitous special explanation to government authorities or private individuals concerned in the farm or garden produce.

Assistance in the collection of practical publications on plant protection organization of public lectures, systematic communication by broadcasting of actual phytosanitary questions.

Publication of an Annual containing the results of the Government Phytosanitary Service.

The organization and rules for the interior management of the Royal Hungarian Phytosanitary Institute will be laid down by a special decree from the Royal Hungarian Minister of Agriculture.

Section 7

The objects of the Royal Hungarian Phytosanitary Service, as the central administrative body, are as under :—

General supervision of phytosanitary affairs of the country, phytosanitary examinations in relation to the international import and export trade in agricultural produce (fruit, potatoes, onions, staddles, vine-shoots, seedlings, slips).

The conducting of examinations on the ground and the taking of measures in case of phytosanitary prohibition ; phytosanitary control and the inspection of establishments concerned with the cultivation, improvement, and trade in agricultural and garden produce (nurseries, seed depots, horticultural establishments, and other establishments for improvement of produce) ; the supervision of the enforcement of compulsory protective measures (extirpation) against insects and harmful plants ; Examination of Phytosanitary control as regards improved plants (seeds, bulbs) and with the verification and registration of the kinds permitted by the State ; compilation and preparation of statistical returns on damage caused by inclemency of the weather, insects and harmful plants, based on official phytosanitary reports ; instruction of district officials and directors, as well as personnel employed on phytosanitary experiments. Encouragement and direction of propaganda for the protection of plants against harmful insects and parasitic plants ; control of the special activity of District Phytosanitary Administrators and examining bodies, and the compilation of statistics on their actions, assistance in the publication of the Government Phytosanitary Service Annual and composition of practical manuals.

The central office of the Royal Hungarian Phytosanitary Service is Ministry of Agriculture V, Kossuth Lajoster 11, Budapest. Telegraphic address ; Plantval Budapest.

The organization and rules for the interior management of the Royal Hungarian Phytosanitary Service will be established by a special decree from the Minister of Agriculture.

Section 8

The Phytosanitary Districts, in their capacity as exterior administrative bodies, have the following tasks :—

Constant supervision of the phytosanitary condition of their area ; the conducting of phytosanitary experiments and qualifications ; reports to the Royal Hungarian Phytosanitary Service of the extent of damage caused by

insects, harmful plants, or the inclemency of the weather ; the commencement of practical and necessary work of a local character on phytosanitary affairs either on their own initiative or in accordance with orders received ; to stimulate with the above objects, local authorities in the enforcement of laws and decrees, and the putting into practice of the required measures ; advice to these authorities ; the submission of proposals to higher authority. Explanation and advice to agricultural producers, voluntarily and on demand ; intermittently, the arrangement of public lectures ; and finally the development of the greatest effort in the cause of phytosanitation within their sphere of activity.

Furthermore, it is their duty to direct and control any work of local phytosanitary agents, to develop their technical knowledge, and to keep themselves acquainted with their reliability and good will, and to prepare in the course of the above a general report summing up the detailed reports of these officials (Service of Information).

They are to correspond with regard to their reports, requests, and proposals direct with the Royal Hungarian Phytosanitary Service in cases of emergency or when circumstances are those of imminent danger ; information can be sent direct to the Royal Hungarian Minister of Agriculture by telegraph or telephone.

The organization of Royal Hungarian Phytosanitary districts is as below :—

Royal Hungarian phytosanitary districts are organized in administrative areas, in the centre of which, or close thereto, should be a winter school of agriculture, a special agricultural school, a secondary school of agriculture, a school of vine cultivation or cenology, or other public instructional establishment in agriculture or horticulture. Special districts are organized in towns with a municipality in big agricultural centres with large areas of cultivated ground from the agricultural and horticultural point of view.

District Directors are chosen from school or Agricultural Institute professors, selected by the Royal Hungarian Minister of Agriculture for instructing on the corresponding subject or from Phytosanitary Service, personnel employed in schools or other public agricultural establishments. Any areas not yet possessed of such a school or establishment will be provisionally attached to the nearest district.

Section 9

Local phytosanitary agents, as employees in the local Government administration, have the following objects :—

The encouragement of the special activities of district phytosanitary directors, and to place in hand work of local phytosanitary interest.

The organization of local phytosanitary agents is as below :—

Local phytosanitary agents are nominated yearly by the Royal Hungarian Minister of Agriculture from agriculturists, vine dressers and gardeners of suitable education. Their range of action should extend over the whole or a part of the area of a town, parish, or parish area, as may be suitable to Royal Hungarian phytosanitary districts.

Section 10

Information Service

To ensure the working of the Government Phytosanitary Service, its information service should be developed to a high degree of efficiency. The information service is both normal and special.

Normal communications include all about damage caused by inclemency of the weather, the importance and devastations of harmful insects and the best known parasitic plants. In accordance with the character of the damage or the agents that cause it, these communications should take place at intervals of varied length.

Special communications take place whenever certain pests, known or unknown, act unexpectedly on a large scale. Such communications should be incidental.

Section 11

The collaboration of Civil Authorities in phytosanitary matters.

The directing agents of the Government Phytosanitary Service should maintain close co-operation with the civil authorities involved, and should be at the disposal of the latter as regards their special opinion.

In return the civil authorities should guarantee uninterrupted work to members of the Government Phytosanitary Service.

Section 12

The task of the Government Phytosanitary Service in the realms of international affairs is as below :—

To ensure the fulfilling of their obligations as regards international trade in plants, and also obligations foreshadowed in the clause of Art. XVII of 1936 on the subject of the insertion of the International Agreement of Rome, dated April 16th, 1929, on plant protection, as well as those foreshadowed in other laws and decrees with regard to plant protection (section 2) and those required by foreign states in international trade of this nature.

Phytosanitary examinations laid down by international agreements in export and import traffic in farm produce are carried out by the Royal Hungarian Phytosanitary Service, and by special agents nominated by the Royal

Hungarian Minister of Agriculture. By the help of these specialists, export produce is graded, and marked with qualification signs. Any attestations related to this subject should be made in the name of " Royal Hungarian Phytosanitary Service."

Section 13

The present decree on the subject of international trade in plants will come into force on January 1st, 1937, and as regards internal trade in November 1st, 1936. Consequently, Decree No. 49,000/1932 F. M. in the rules of the Royal Hungarian Service for the protection of plants becomes null and void as regards international trade in plants, with effect from January 1st, 1937, and as regards internal trade with effect from November 1st, 1936.

Ferenc MARSCHALL, Dr.
m.p.
Secretary of State,
(for Minister).

BUDAPEST ;
October 27th, 1936.

LIST OF SPECIALIST OFFICIALS AUTHORIZED TO SIGN PHYTOSANITARY CERTIFICATES AS LAID DOWN BY THE GOVERNMENT PHYTOSANITARY SERVICE IN THE KINGDOM OF HUNGARY.

MARTON ACZEL	Official in special employment.
LASZLO AGH	Agriculturist with diploma. (Official in special employment.)
GUSZTAV AIGNER. . . .	Director of Royal Hungarian Special School of Agriculture.
JANOS AMBRUS	Professor of Agriculture. (Delegated director.)
ISTVAN BAGOTAI	Supernumerary Agricultural Official. (Delegated assistant.)
GABOR BAKO	Director General of Experimental Agricultural Research.
LASZLO BALAZSOVICH . . .	Agriculturist with diploma. (Official in special employment.)
PAL BALPATAKY	Supernumerary Official in Vine Cultivation.
ISTVAN BARRA	Supernumerary Official in Experimental Agricultural Research.
FERENC BARANYOVITS . .	Supernumerary Official in Experimental Agricultural Research.
JOZSEF BENEDEK	Professor of Agriculture.
JOZSEF BONA	Sub-professor of Agriculture.
ENDRE BORY	Inspector of Agriculture (retired).
LASZLO CZAJLIK	Sub-professor of Royal Hungarian Agriculture.

Aladar Cziraky	Agriculturalist with diploma (in special employment).
Istvan Csabai	Supernumerary Agricultural official.
Bela Csanady	Professor of Agriculture (retired.)
Jeno Cseh	Sub-professor of Agriculture.
Lajos Cserey	Inspector of Horticulture.
Endre Csete	Professor of Agriculture.
Sandor Csete	Chief-assistant in Experimental Agricultural Research.
Gyorgy Csongradi	Professor of Agriculture (delegated director).
Zoltan Csorba	Supernumerary official in experimental agricultural research.
Beno Diera	Inspector General of Horticulture (director).
Istvan Dobo	Sub-professor of Agriculture.
Erno Dravetzky-Koos	Professor of Agriculture.
Tibor Erdelyi	Professor of Agriculture.
Andras Ferke	Horticulturalist with diploma (in special employment).
Laszlo Gajary	Professor of Agriculture (delegated director).
Istvan Galamb	Inspector General of Vine Cultivation.
Denes Gabos	Professor of Agriculture (delegated director).
Janos Gaman	Sub-professor of Agriculture.
Ifj. Istvan Gergely	Sub-inspector of Horticulture.
Janos Gergely	Horticulturalist with diploma (in special employment).
Zoltan Gero	Assistant in Experimental Agricultural Research.
Janos Giber	Professor of Agriculture (delegated director).
Janos Gollner	Assistant in the Royal Hungarian Academy of Agriculture.
Ferenc Gruber	Supernumerary Agricultural Official.
Ferenc Gucsy	Sub-professor of Agriculture (delegated director)
Antal Gulyas	General professor in the Royal Hungarian Academy of Agriculture.
Kornel Gyenes	Sub-inspector of Vine Cultivation.
Gyula Hajos	Inspector of Horticulture.
Istvan Hajos	Supernumerary Agricultural Official.
Gyula Halmos	Supernumerary Official of Vine Cultivation.
Istvan Harkai	Professor of Agriculture.
Erno Hemela	Sub-inspector of Vine Cultivation.
Kalman Hinfner	Supernumerary Official in Experimental Agricultural Research.
Vitez Ference Horvath	Chief Chemist in Experimental Agricultural Research.
Vitez Ivan Horvath	Professor of Agriculture (delegated director).
Sandor Imre	Professor of Agriculture (delegated director).

Andor I'so	Agriculturalist with diploma (supernumerary official).
Istvan Janko	Inspector of Vine Cultivation.
Viktor Kaczian	Agriculturalist with diploma (in special employment).
Gyula Kacsmarik	Professor of Agriculture.
Gyula Kadocsa	Director of Experimental Agricultural Research.
Geza Kapitany	Professor of Agriculture (delegated director).
Gyorgy Kelecsenyi	Sub-professor of Agriculture.
Hermann Kern	Director General of Experimental Agricultural Research, and member of upper council of rural economy.
Laszlo Ketter	Supernumerary Agricultural Official.
Gyorgy Komlossy	Assistant in Experimental Agricultural Research.
Viktor Kosinszky	Supernumerary official in experimental agricultural research.
Zoltan Kovacs	Director of Royal Hungarian Special School of Agriculture.
Jozsef Kranitz	Inspector General of Horticulture.
Jozsef Krenner	Chief Assistant in Experimental Agricultural Research.
Ferenc Kriszta	Sub-inspector of Horticulture.
Aladar Laczko	Chief Assistant in Experimental Agricultural Research.
Vitez Bela Lomjapataky	Inspector General of Vine Cultivation.
Bela Ludany	Sub-professor of Agriculture.
Gabor Maroti	Supernumerary Agricultural Official.
Denes Marton	Inspector General of Vine Cultivation.
Ifj. Peter Matusovits	Agriculturalist with diploma, in sepcial employment.
Janos Matrai	Professor of Agriculture.
Sandor Matray	Agriculturalist with diploma (administrator of Farm land cultivation).
Imre Meiszner	Inspector of Vine Cultivation.
Gyula Meszoly	Horticulturalist with diploma.
Jozsef Mikes	Supernumerary Agricultural Official.
Bela Mocsy	Supernumerary Agricultural Official.
Laszlo Molnar	Inspector of Agriculture (retired).
Aladar Neth	Professor of Agriculture.
Jeno Nemeth	Professor of Agriculture.
Zoltan Nemethy	Director of Royal Hungarian Special School of Agriculture.
Ivan Okalyi	Inspector of Horticulture.
Miklos Orincsay	Sub-professor of Agriculture.
Gyula Orosz	Professor of Agriculture (delegated director).

Lajos Palvolgyi	Supernumerary Agricultural Official.
Bela Pettenkoffer	Agriculturalist with diploma, in supernumerary employment.
Vitez Ferenc Peterdy	Professor of Agriculture (delegated director).
Laszlo Gyorgy Polgar	Inspector of Horticulture.
Janos Regele	Sub-professor of Agriculture.
Dezso Revy	Special professor of the Royal Hungarian Agricultural Academy.
Lajos Ripka	Inspector of Horticulture.
Laszlo Santha	Chief Assistant in Experimental Agricultural Research.
Gyorgy Sarospataki	Agriculturalist with diploma in special employment.
Jeno Sebestyen	Professor of Agriculture (delegated director).
Istvan Sebeok	Professor of Agriculture.
Sandor Seemann	Professor of Agriculture (delegated director).
Frigyes Seiffert	Specialist Official.
Jeno Suhajda	Sub-professor of Agriculture.
Miklos Szabo	Auxiliary Manager of Horticulture.
Jozsef Szajbely	Special Professor at the higher school of Horticulture.
Gusztav Szelenyi	Assistant in Experimental Agricultural Research.
Lajos Szentgyorgyvari	Director of Royal Hungarian Special School of Agriculture.
Lazar Szentirmay	Control Official.
Geza Szent-Galy-Faur	Supernumerary Agricultural Official.
Dezso Szilagyi	Inspector of Vine Cultivation.
Jeno Sziranyi	Inspector General of Vine Cultivation.
Janos Szirmai	Agriculturist with diploma (in special employment).
Endre Szlavik	Inspector of Horticulture.
Bela Szombath	Professor of Agriculture (delegated director).
Ifj. Kalman Tabajdi	Chief Assistant in Experimental Agricultural Research.
Gyorgy Terray	Supernumerary Vine Cultivation Official.
Agoston Toth	Supernumerary Agricultural Official.
Janos S. Toth	Sub-professor of Agriculture.
Istvan Urbanyetz	Supernumerary Official.
Jeno Urbanyi	Assistant in Experimental Agricultural Research.
Miklos Yadanyi	Sub-professor of Agriculture.
Jeno Vadnai	Professor of Agriculture (delegated director).
Sandor Vancsay	Professor of Agriculture.
Geza Varga	Professor of Agriculture (director).
Antal Varkonyi	Inspector of Vine Cultivation.

Gyula Vegh	Director of Royal Hungarian Special School of Agriculture.
Lajos Veghelyi . . .	Chief Assistant in Experimental Agricultural Research.
Gyula Veniss	Professor of Agriculture (delegated director.)
Istvan Vigh	Chief Assistant in Experimental Agricutural Research.
Bela Weitner	Agriculturalist with dipoma, in special employment.
Jeno Gyorffy	Chief Assistant in Experimental Agricultural Research (retired).
Kornel Kallay. . . .	Sub-professor of Agriculture.

REVIEWS

Soilless Growth of Plants. By Carleton Ellis and Miller W. Swaney. Reinhold Publishing Corporation, 330 West, 42nd Street, New York, N. Y. (1938). Pp. 155. Price $2·75 net.

The extremely interesting subject—soilless growth of plants, that is, the art of growing plants without soil—has so long been merely a matter of scientific interest and scattered information on this subject could be had only from scientific publications which ordinarily are neither very accessible nor intelligible to a layman. The growing of plants in culture solution is well known to students of plant physiology and only very recently there has been a move on the part of a few enterprising gardener-scientists to exploit the commercial possibility of the technique. In recent years brief articles of a popular nature on this subject have occasionally appeared in the leading papers of Great Britain and the United States; but to the most people these are yet nothing but a sensational piece of news of somewhat doubtful utility.

Messrs. Ellis and Swaney whose contributions to the development of the soilless technique are well known, have attempted for the first time to place before the general public the details of growing plants without soil. It is the first complete treatise which discusses thoroughly the three important methods of soilless growth—water culture, sand culture and sub-irrigation. In the present work they have expressed in popular language all the relevant information on the subject for the use of horticulturists and amateur gardeners. In eight chapters they have surveyed the whole process of soilless growth of plant, beginning from a general discussion on the chemistry of plant life to the formulae of nutrient solutions which are required for plant metabolism. The subject matter of the book is likely to appeal both to laymen and scientists, specially because the authors have not shown any tendency to indulge in high-sounding schemes, more sensational than practical, but have presented the subject as a scientifically sound and workable method.

Wherever there is too much pressure on the land and the climatic conditions are adverse to the production of green fodders and flowers all the year round, the technique of soilless plant growth will open up new possibilities. These methods will, therefore, undoubtedly prove attractive to American and Continental gardeners or farmers, specially in such localities where greenhouse raising of flower plants and vegetable is a commercial proposition. For obvious reasons, the ultimate value of the methods described in the book will be very limited, almost negligible, under present Indian conditions.

Although the underlying principles, as detailed by the authors, are equally of interest both to the layman and the scientist, a word of caution is necessary regarding the universal application of the methods. Different species of

flowers or vegetables have not the same nutrient requirements, and for efficient growth and development every species should have its specific nutrient. It is, therefore, somewhat hazardous to treat all types of vegetation by standard methods. Unfortunately, nowhere in this book have the authors clearly drawn attention to this fact, nor to the fact that considerable amount of work yet remains to be done before the methods described could be widely applied. In their attempt to present facts in non-technical language, the authors have naturally steered clear of discussions of a technical nature, which detracts much from the real value of the book to scientific readers.

Altogether the book is likely to catch popular imagination and the authors have successfully unmasked the ' mystery ' of growing plants without soil.

There are in the book certain loose expressions like ' rest period in plant ', which seems to have been applied with a view to appeal to the imagination of non-technical readers. In such publications scientific accuracy or discourses of highly technical nature are not always possible, as that would, as apprehended by the authors, ' tend only to confuse the reader or cause him to lose interest.'

The printing and the general get-up of the book are excellent. (B. K. M.)

Plant Injection for Diagnostic and Curative Purposes (*Technical Communication 10 of the Imperial Bureau of Horticulture and Plantation Crops, East Malling, Kent, England*). By W. A. Roach. Foreword by Prof. V. H. Blackman : 1938, pp. 78, plates 2, text-figures 47, bibl. 162. Price 5*s*.

Man has toyed for centuries with the idea of feeding his fruit trees direct instead of by more orthodox but slower methods and in the heyday of the Italian Renaissance we find Leonardo da Vinci noting that if it is desired to produce poisoned fruit the injection of fruit trees offers a way.

More recently various ' tree doctors ' have suggested that easy rejuvenation of orchards can be effected by the use of their own particular injection methods, but such methods have always been somewhat suspect, since obviously different conditions demand different remedies and the cure may prove worse than the disease.

It has remained for Dr. Roach to bring order out of chaos and show those of us who are attracted by the idea how to set about our investigations.

He starts by giving a history of injection work in Europe and the U. S. A., paying particular attention to that published in recent years on the detection and cure of deficiency diseases. He notes also the successful use of injection for purely physiological purposes, as, for instance, of glucose into vines, just before bud break, to ensure a good fruit set. He points out, moreover, how

injection methods may be of great value in the study, not only of mineral deficiencies, but also of rootstock influence and of the effect of the chemical constituents of a fruit on its storage capacity.

Recent cases of successful injection cited include the following diagnosis of incipient chlorisis of the iron shortage type at East Malling; diagnosis and cure of a copper deficiency dieback disease in apples in Western Australia; determination of the cause and cure of boron deficiency diseases of apples in Canada and New Zealand; increase in vigour and freedom from insect pests in apple trees injected with various solutions in England.

His own experiments have been in progress for seven years on material which has ranged from the strawberry plant to fully grown apple and plum trees, and most of the present paper is devoted to a consideration of the methods used. It is for this clear and illustrated account that horticulturist will be particularly grateful. He describes in detail with the help of text-figures and lists of tools the technique used for the injection of particular parts of standing trees and other plants, varying in size from a single interveinal area of a leaf to whole main branches, noting, moreover, the type of problem for which each particular method is best suited. He shows how in the most delicate methods the injection of leaves and their comparison with neighbouring un treated leaves or parts of leaves enables a rapid diagnosis to be made of the mineral deficiency from which a plant or tree is suffering. He describes how whole trees may be injected for experimental purposes or even for economic reasons in the commercial orchard.

In short he shows us how to carry out the operations advocated and how to avoid those many pitfalls which await the over-zealous, although he is far from suggesting that all his methods are perfect.

He invites criticism and the experience of others in the same field.

RECENT PUBLICATIONS OF THE IMPERIAL AGRICULTURAL BUREAUX

Annual Reports of the Executive Council of the Imperial Agricultural Bureaux

List of Agricultural Research Workers in the British Empire, 1937*

OBTAINABLE FROM THE SECRETARY, EXECUTIVE COUNCIL, IMPERIAL AGRICULTURAL BUREAUX, 2, QUEEN ANNE'S GATE BUILDINGS, LONDON, S. W. 1, AND FROM H. M. STATIONERY OFFICE, LONDON, W. C. 2

The Journal of Dairy Research

Published by the Cambridge University Press on behalf of the Committee of Management. First issued in November, 1929.

	s.	d.
Volumes I to IV (each two parts)	25	0
Single parts	15	0
Volumes V to IX (each three parts)	25	0
Single parts	10	0

I. OBTAINABLE FROM THE IMPERIAL BUREAU OF SOIL SCIENCE, ROTHAMSTED EXPERIMENTAL STATION, HARPENDEN, HERTS

Soils and Fertilisers

A comprehensive abstract journal of soil science published six times yearly.

	British Commonwealth s.	British Commonwealth d.	Foreign s.	Foreign d.
Ordinary edition	20	0	25	0
Special edition (printed one side only)	25	0	30	0
Both editions	40	0	50	0
Single copies, ordinary edition only	5	0	5	0

Discontinued Series

The following series of multigraphed periodicals have now been incorporated in Soils and Fertilisers. Annual sets (some incomplete) are obtainable at the prices given below.

Publications relating to Soils and Fertilisers (monthly) 1935 (8 issues) 6*s.*; 1936 (10 issues) 7*s.* 6*d.*; 1937 (10 issues) 7*s.* 6*d.*

Monthly Letters. 1935 (10 issues); 1936 (12 issues); 1937 (11 issues) 4*s.* per annum.

Recent Developments in Soil Analysis (quarterly), Complete Sets, 8*s.*

* 1938 Edition in preparation.

Occasional Papers

	s.	d.
Technical Communications—		
37. Soil Structure, by E. J. Russell	2	0
Annual Report for the years 1929-30 to 1937-38 . . . each	0	6
Bibliographies—		
Bibliography on Coffee	2	0
Catalogue of Journals and Periodicals in the Library of Rothamsted Experimental Station	2	0
Special Publication—		
The Katamorphism of Igneous Rocks under Humid Tropical Conditions by the late Sir J. B. Harrison	5	0
Bibliography of Soil Science, Fertilizers and General Agronomy, 1934-37	25	0

II. Obtainable from the Imperial Bureau of Animal Health, Veterinary Research Laboratory, New Haw, Weybridge, Surrey

Abstracting Journal

	s.	d.
The Veterinary Bulletin—		
1931. Vol. I. Quarterly (1st Number, April)	7	6
Annual subscription	20	0
Subsequent Volumes. Monthly (1st Number, January)	5	0
Annual subscription (postage paid) (Back numbers still available)	40	0

Indexing Publication

Index Veterinarius. First issue April, 1933. Annual subscription (postage paid). Volumes I to III mimeographed, Volume IV onwards printed (Vols. I—V issued quarterly. Vol. VI onward issued half yearly) (Back numbers still available).	100	0

Occasional Papers

Review series—		
No. 1. Bovine Mastitis by E. M. Peterson	10	0

III. Obtainable from the Imperial Bureau of Animal Nutrition, Rowett Research Institute, Bucksburn, Aberdeen

Journal

	s.	d.
Nutrition Abstracts and Reviews. (Issued under the direction of the Imperial Agricultural Bureaux Council, the Medical Research Council and the Reid Library)		
Subscription per Volume of 4 numbers	42	0
Per single number	13	0

Occasional Papers

Technical Communications—		
10. Table of Composition of Foods giving Energy, Value, Protein, Fat, Carbohydrate, Calcium, Phosphorus and Iron Content of Some 300 Foods Commonly used in Great Britain and Compiled for Use in the Analysis of Dietary Surveys	1	0
Occasional Communications—		
2. Etiology of Endemic Goitre		

IV. Obtainable from the Imperial Bureau of Plant Breeding and Genetics, Plant Breeding Institute, School of Agriculture, Cambridge

Journal

	s.	d.
***Plant Breeding Abstracts*—**		
Quarterly. Annual Subscription	*25	0
Single copy	7	6
Volume I (Nos. 1, 2 and 3 out of print), No. 4 available	7	6
„ II Out of print	..	
„ III (Nos. 1, 2 and 4 out of print), No. 3 available	..	
„ IV (Nos. 1, 2 and 4 out of print), No. 3 available	7	6
„ V (No. 2 out of print), Nos. 1, 3, 4 available	17	6
„ VI (complete)	20	0
„ VII (complete)	20	0
Numbers printed on one side of paper	30	0

Occasional Papers

Indexes to Plant Breeding Abstracts—		
Subject Index to Vols. I to V of Plant Breeding Abstracts	5	0
Subject Index to Vols. VI and VII of Plant Breeding Abstracts	2	6
Supplements to Plant Breeding Abstracts—		
Summary of Reports received from Countries exclusive of the British Empire, 1928-31. Supplement I	2	6
Summary of Reports received from Stations in the British Empire, 1932-35. Supplement II	5	0
Technical Communications—		
Vernalization and Phasic Development of Plants (Joint Publication with the Imperial Bureau of Pastures and Forage Crops)	10	0
Bibliographical Monographs—		
Tobacco Breeding Bibliography	1	0

V. Obtainable from the Imperial Bureau of Pastures and Forage Crops, Welsh Plant Breeding Station, Agricultural Buildings, Alexandra Road, Aberystwyth, Wales

Journal

	s.	d.
***Herbage Abstracts*—**		
Vol. 7, 1937 (printed both sides)	15	0
Vol. 7, 1937 (printed one side only)	20	0
Vol. 8, 1938 (published price)	25	0
Vol. 8, 1938 (Price in Great Britain and British Commonwealth)	20	0
Herbage Reviews—		
Vol. 5, 1937	10	0
Vol. 6, 1938	†15	0

* 20*s.* to subscribers in the British Commonwealth.

† A reduction of 5*s.* will be given when Herbage Abstracts is also ordered.

Occasional Papers

	s.	d.
Bulletin—		
Erosion and Soil Conservation*	5	0

VI. Obtainable from the Imperial Bureau of Horticulture and Plantation Crops, East Malling Research Station, East Malling, Kent

	s.	d.
Journal		
Horticultural Abstracts. A quarterly abstract publication of current horticultural literature.		
Vol. I. First issued, March, 1931. No. 2, single copy . . .	1	6
Vol. II. Nos. 1, 2 and 3 out of print. No. 4, single copy . . .	1	6
Vols. III to VII, each (single copy 4*s.*)	15	0
Vols. VIII and subsequent volumes. Annual subscription . . .	†25	0
Single copy	6	6
Technical Communications		
10. Plant Injection for Diagnostic and Curative Purposes. 1938. W. A. Roach	5	0
Occasional Papers		
5. Frameworking of Fruit Trees. R. J. Garner and W. F. Walker .	1	0
Proceedings of First Imperial Horticultural Conference, 1930—		
Part II. Papers on the application of Science to Horticulture. 1931. 2*s.* Out of print, but the following separates are available, each .	0	3
Field Experiments in Certain Tropical and Sub-Tropical Crops in West Africa (Dr. E. J. Martin and W. H. Beckett).		
Fruit Products and Associated Problems (Professor B. T. P. Barker).		
Some Physiological Considerations in Horticulture (Professor V. H. Blackman).		
Plant Physiological Work in the Tropics: Some of the Problems, with Special Reference to Cocoa and some Possible Lines of Attack (Dr. T. G. Mason and Dr. E. J. Maskell).		
Soil and Survey Work as a Basis for Fruit Production in Irrigated Areas (Professor A. J. Prescott).		
	s.	d.
Part III. Papers on Progress in Fruit Storage Methods, 1931, 2*s.* 6*d.* Out of print, but the following separates are available, each . . .	0	3
Factors Influencing the Storage Qualities of Fruit (Dr. T. Wallace).		
The Present Position of the Bitter-Pit Problem in Australia (W. M. Carne, H. A. Pittman and H. G. Elliott).		
The Behaviour and Diseases of the Banana in Storage and Transport (Dr. C. W. Wardlaw and Dr. L. P. McGuire).		
Problems of Storage and Transport (E. A. Griffiths).		
Fruit Transport Problems in Canada (R. L. Wheeler).		

---

* Joint publication of bureaux of Soil Science and Pastures and Forage Crops.

† 20*s.* a volume or 5*s.* a number to subscribers in the British Commonwealth.

The Infection and Invasion of the Apple Fruit by Fungi in Relation to the Problem of Storage (Dr. A. S. Horne).

Experiments on the Preservation of Citrus Fruits (Dr. W. J. Young and F. M. Read).

Other Publications—

Index to Volumes I—X of the *Journal of Pomology and Horticultural Science*, 1933. Compiled by Bureau, published by the Editors of the *Journal of Pomology and Horticultural Science.*

	s.	d.
Available from the Bureau	5	0
Old and new standpoints on senile degeneration, 1931. By A. P. C. Bijhouwer	0	6

VII. OBTAINABLE FROM THE IMPERIAL BUREAU OF ANIMAL BREEDING AND GENETICS, INSTITUTE OF ANIMAL GENETICS, UNIVERSITY OF EDINBURGH, KINGS' BUILDINGS, WEST MAINS ROAD. EDINBURGH

Journal	s.	d.
Animal Breeding Abstract (quarterly) from Vol. 6 (1938)—		
Annual subscription	25	0
Subscribers in the British Commonwealth	20	0
Earlier Vols. from 1933, Vols. I—V. Annual subscription . . .	15	0
Occasional Papers		
Animal Breeding in the British Empire. A Survey of Research and Experiment, 1934	2	0
Also obtainable from the Bureau:		
The Genetics of the Horse, 1930. Reprinted from 'Bibliographia Genetica'	0	6
Inheritance of Productivity in Farm Stock, 1935. Reprinted from 'Empire Journal of Experimental Agriculture'	0	6
The Genetics of the Pig, 1936. Reprinted from 'Bibliographia Genetica'	2	6

VIII. OBTAINABLE FROM THE IMPERIAL BUREAU OF AGRICULTURAL PARASITOLOGY (HELMINTHOLOGY), INSTITUTE OF AGRICULTURAL PARASITOLOGY, WINCHES FARM DRIVE, HATFIELD ROAD, ST. ALBANS, HERTS

Journal	s.	d.
Bibliography of Helminthology. For the years 1930, 1931, 1932, 1933— Library Edition, bound in cloth per volume	10	6
Stiff paper cover only, per volume	8	0
Helminthological Abstracts (1932 onwards). Issued in five or six parts per volume, each volume covering the literature published in a single year. Subscription per volume (payable in advance) . . .	30	0
Complete volumes bound in cloth	32	0

[NOTE.—From Vol. III (1934) this journal incorporates the *Bibliography of Helminthology* and is published by the Bureau instead of by the *Journal of Helminthology*.]

Other Publications		
(*j*) Helminth parasites of New Zealand. By May R. Young, 19 pp.. .	4	0
Notes and Memoranda—		
11. Recent Developments in the Control of Heterodera marioni, 6 pp. .	1	0

PUBLICATIONS ISSUED BY THE IMPERIAL INSTITUTE OF ENTOMOLOGY, 41, QUEEN'S GATE, LONDON, S. W. 7

Bulletin of Entomological Research—

Published quarterly, and containing original articles on Economic Entomology. Issued post free.

	s.	d.
Annual Subscription (payable in advance)	30	0
Subscription to current volume received after 30th June	36	0
Separate parts	9	0
Back Volumes—I (1910)—X	20	0
XI (1920)—XXIII (1932)	25	0
XXIV (1933) *et seq.*	37	6
Bound Volumes (extra to above prices)	6	0
Binding cases only	3	6

Reprints of some of the papers published in this Journal are available. Prices on application.

Review of Applied Entomology—

Consisting of abstracts or reviews of all current literature on Economic Entomology throughout the world. Published monthly in two series :—

Series 'A' dealing with insect and other Arthropod pests of cultivated plants, forest trees and stored products of animal and vegetable origin.

Series 'B' dealing with insects, ticks, etc., conveying disease or otherwise injurious to man and animals. Issued post free.

	Series 'A' s.	Series 'A' d.	Series 'B' s.	Series 'B' d.
Annual Subscription (payable in advance)	30	0	15	0
Subscription to current volume received after 30th June	36	0	18	0
Separate parts	3	0	1	6
Annual Subject Index (when sold separately)	6	0	3	0
Subject Index—Vols. I—III	5	0	I—X10	0
Back Volumes—Vols. I (1913)—VII, per volume	15	0	10	0
Vols. VIII (1920)—XXII (1934), per volume	24	0	12	0
Vol. XXIII (1935) *et seq.* per volume	42	0	21	0
Bound Volumes (extra to above prices)	5	0	5	0
Binding cases only	3	6	3	6

The index to the current volume is not published until April (Series 'B') or August (Series 'A') of the following year.

Zoological Record—Part Insecta—

Published annually about July in each year and containing as complete a record as possible of the literature of the previous year, chiefly from the systematic standpoint.

	s.	d.
Annual Subscription (including postage)	15	6
Back Volumes—Vols. LII (1915)—LVII (1920)	12	0
Vol. LVIII (1921) *et seq.*	15	0

Postage extra.

Some of the Sections into which Volumes LXI—LXVII were divided are still available. Prices on application.

The Biological Control of an Insect in Fiji—

By T. H. C. Taylor, M.Sc. (Lond.). An account of the Coconut Leaf-mining Beetle and its Parasite Complex. Royal 8 vo. pp. X and 239 with 23 plates, 2 maps and 17 text figures. Bound in cloth. Price 12*s.* Postage, inland, 6*d.*; abroad, 10*d.* 1937.

Report of the Fourth Imperial Entomological Conference, 19th—27th September, 1935.

Royal 8 vo. 70 pp. Paper covers. Price, 4*s.* 0*d.* 1935.

Orders may be sent direct to the Assistant Director, Imperial Institute of Entomology, 41, Queen's Gate, London, S. W. 7, or through a bookseller.

PUBLICATIONS OBTAINABLE FROM THE IMPERIAL MYCOLOGICAL INSTITUTE, KEW, SURREY

Journal

Review of Applied Mycology—

	s.	*d.*
Annual Subscription, 12 monthly parts, with title-page and index (post free)	*30	0
Single part	3	0
Title-page and index	4	0

Back volumes (with the exception of Vols. I, VII and VIII, which are out of print) can be supplied, postage extra.

Occasional Publications

Annotated Account of Fungi Received at the Imperial Mycological Institute.

	s.	*d.*
List I	1	0
List II, Fasc. 1	1	0
List II, Fasc. 2	2	0
List II, Fasc. 3. General Part	1	0
Report on the Third Imperial Mycological Conference, 1934	2	0
Mycologists resident in the British Empire, 1938	1	0

* 24*s.* to subscribers in the British Commonwealth, who send their subscription direct to the Institute.

PUBLICATIONS OF THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH, INDIA

[Prices are inclusive of packing and Indian Postage]

1. Agriculture and Live-stock in India

ICAR. 1. A bi-monthly journal of agriculture and animal husbandry for the general reader interested in agriculture or live-stock in India or the Tropics. (Established 1931. Published in January, March, May, July, September and November. Prepayable subscription Rs. 6 or 9*s*. 9*d*. per annum. Price per part Rs. 2 or 3*s*. 6*d*.). Volumes I to VIII complete are available.

2. The Indian Journal of Agricultural Science

ICAR. 5. A bi-monthly scientific journal of agriculture and the allied sciences, mainly devoted to the publication of the results of original research and field experiments. (Established 1931. Published in February, April, June, August, October and December. Prepayable subscription Rs. 15 or 24*s*. per annum. Price per part Rs. 3 or 5*s*. 3*d*.). Volumes I to VIII complete are available.

3. The Indian Journal of Veterinary Science and Animal Husbandry

ICAR. 6. A quarterly journal for the publication of scientific matter relating to the health, nutrition and breeding of live-stock. (Established 1931. Published in March, June, September and December. Prepayable subscription Rs. 6 or 9*s*. 9*d*. Price per part Rs. 2 or 3*s*. 6*d*.). Volumes I to VIII complete are available.

4. Scientific Monographs of the Imperial Council of Agricultural Research

ICAR. 10·1 No. 1. The Fungi of India. By E. J. Butler, C.I.E., D.Sc., M.B., F.R.S. and G. R. Bisby, Ph.D. (1931). Price Rs. 6-12-0 or 11*s*.

ICAR. 10·2 No. 2. Life-histories of Indian Microlepidoptera, Second Series: Alucitidæ (Pterophoridæ, Tortricina and Gelechiadæ). By T. Bainbrigge Fletcher, R.N., F.L.S., F.E.S., F.Z.S. (1932). Price Rs. 3-4-0 or 5*s*. 6*d*.

ICAR. 10·3 No. 3. The Open Pan System of White-Sugar Manufacture. By R. C. Srivastava, B.Sc. (1935, 2nd Edition.) Price Rs. 3-2-0 or 5*s*. 0*d*.

ICAR. 10·4 No. 4. Life-histories of Indian Microlepidoptera; Cosmopterygidæ to Neopseustidæ. By T. Bainbrigge Fletcher, R.N., F.L.S., F.E.S., F.Z.S. (1933). Price Rs. 4-8-0 or 7*s*. 6*d*.

ICAR. 10·5 No. 5. The Bombay Grasses. By E. J. Blatter, S.J., Ph.D., F.L.S. and C. McCann, F.L.S. Illustrated by R. K. Bhide. (1935). Price Rs. 20-12-0 or 32*s*. 6*d*.

ICAR. 10·6 No. 6. Helminth Parasites of the Domesticated Animals in India. By G. D Bhalerao, M.Sc. (1935) Price Rs. 7-12-0 or 13*s*. 3*d*.

ICAR. 10·7 No. 7. Influence of Manures on the Wilt Disease of *Cajanus indicus* Spreng. and the Isolation of Types Resistant to the Disease. By W. McRae, M.A., D.Sc. (Edin.), F.L.S. and F. J. F. Shaw, D.Sc. (Lond.), A.R.C.S., F.L.S. (1933). Price Rs. 2-4-0 or 4*s*. 3*d*.

ICAR. 10·8 No. 8. The Silk Industry of Japan with Notes on Observations in the United States of America, England, France and Italy. By C. C. Ghosh, B.A., F.E.S. (1933). Price Rs. 4 or 6*s*. 9*d*.

ICAR. 10·9 No. 9. Mechanical Cultivation in India. A History of the Large-scale Experiments carried out by Burmah-Shell Oil Storage and Distributing Company of India, Ltd. By C. P. G. Wade. (1935). Price Rs. 3-14-0 or 6*s*. 6*d*.

ICAR. 10·10 No. 10. The Spotted Boll Worms of Cotton (*Earias fabia* Stoll and *Earias insulana* Boisd.) in South Gujarat, Bombay Presidency. (Final report on investigations financed by the Indian Central Cotton Committee, 1923-1931). By B. P. Deshpande, M.Ag. and N. T. Nadkarny, B.Ag. (1936). Price Rs. 5-14-0 or 9*s*. 6*d*.

ICAR. 10·11 No. 11. Investigations on the Course and Distribution of the Nerves supplying Levator anguli scapuli and Rhomboideus muscles and the formation of the Phrenic Nerve in the Ox, with Observations on certain Anatomical Deviations. By H. N. Chelva Ayyangar, G.M.V.C. (1937). Price Rs. 4-10-0 or 7*s*. 9*d*.

ICAR. 10·12 No. 12. The Fungi of India, Supplement I. By B. B. Mundkur. Price Re. 1-6-0 or 2*s*. 3*d*.

ICAR. 10·13 No. 13. Further Observations on Anatomical Deviations in the Ox and Notes on certain Anatomical Freaks. By H. N. Chelva Ayyangar, G.M.V.C. (*In Press*.)

5. Miscellaneous Bulletins of the Imperial Council of Agricultural Research

ICAR. 8·1 No. 1. List of Publications on Indian Entomology, 1930. Compiled by the Imperial Entomologist, Pusa. (1934). Price As. 14 or 1*s*. 6*d*.

ICAR. 8·2 No. 2. List of Publications on Indian Entomology, 1931. Compiled by the Imperial Entomologist, Pusa. (1934). Price As. 8 or 10*d*.

ICAR. 8·3 No. 3. List of Publications on Indian Entomology, 1932. Compiled by the Imperial Entomologist, Pusa. (1934). Price As. 12 or 1*s*. 3*d*.

ICAR. 8·4 No. 4. Host Plant Index of Indo-Ceylonese Coccidae. By S. Ramachandran, L.Ag. and T. V. Ramakrishna Ayyar, B.A., Ph.D., F.Z.S. (1934). Price Re. 1-10-0 or 2*s*. 9*d*.

ICAR. 8·5 No. 5. List of Publications on Indian Entomology, 1933. Compiled by the Officiating Imperial Entomologist, Pusa. (1935). Price As. 9 or 1*s*.

ICAR. 8·6 No. 6. Bee-keeping. By C. C. Ghosh, B.A., F.R.E.S. (3rd Edition.) (1936). Price Re. 1-14-0 or 3*s*. 3*d*.

ICAR. 8·7 No. 7. List of Publications on Indian Entomology, 1934. Compiled by the Imperial Entomologist, Pusa. (1935). Price Re. 1-2-0 or 2*s*.

ICAR. 8·8 No. 8. Selected Clinical Articles, Part I. By G. K. Sharma, G.P.V.C. and R. L. Kaura, B.V.Sc., M.R.C.V.S. (1936). Price As. 8 or 10*d*.

ICAR. 8·9 No. 9. Statistical Methods and their Application to Agronomy; A bibliography. Compiled by K. K. Guha Roy, B.A. (1936). Price Rs. 2-2-0 or 4*s*.

ICAR. 8·10 No. 10. Diseases of Sugarcane and Methods for their Control. By L. S. Subramaniam. (1936). Price Re. 1-14-0 or 3*s*. 3*d*.

ICAR. 8·11 No. 11. Tables of Standard Errors of Mendelian Ratios. Compiled by Swarn Singh Purewal, M.Sc., Ph.D. (Cornell) and P. Krishna Rao, L.Ag. (1936). Price As. 12 or 1*s*. 3*d*.

ICAR. 8·12 No. 12. List of Publications on the Botany of Indian Crops, Part II, for the period 1928-32. Compiled by R. D. Bose, B.Sc. (1936). Price Rs. 3-6-0 or 5*s*. 9*d*.

ICAR. 8·13 No. 13. Two New Statistical Tables based upon Fisher's '*t*'. By M. Vaidyanathan, M.A. (1936). Price As. 6 or 8*d*.

ICAR. 8·14 No. 14. List of Publications on Indian Entomology (1935). Compiled by the Imperial Entomologist, Pusa. (1936). Price Re. 1-4-0 or 2*s*.

ICAR. 8·15 No. 15. Selected Clinical Articles, Part II. By G. K. Sharma, G.P.V.C., R. L. Kaura, B.V.Sc., M.R.C.V.S., S. Ganapathy Iyer, G.M.V.C., G. S. Khan, B.Sc., and M. Y. Mangrulkar, M.Sc., M.R.C.V.S., D.T.V.M. (Edin.) (1937). Price Re. 1-4-0 or 2*s*.

ICAR. 8·16 No. 16. Indian Grazing Conditions and the Mineral Contents of some Indian Fodders. By P. E. Lander, M.A., D.Sc., F.I.C., I.A.S. (1937). Price Rs. 3-14 or 6*s*. 9*d*.

ICAR. 8·17 No. 17. A Brief Survey of some of the Important Breeds of Cattle in India. By Col. Sir Arthur Olver, C.B., C.M.G., F.R.C.V.S., F.N.I. (1938). Price Rs. 2 or 3*s*. 6*d*.

ICAR. 8·18 No. 18. Milk Records of Cattle in Approved Dairy Farms in India. By K. P. R. Kartha, B.A. (1938). Price Rs. 2-12-0 or 4*s*. 6*d*.

ICAR. 8·19 No. 19. A Preliminary Annotated List of the Fruit Pests of the North-West Frontier Province. By Hem Singh Pruthi, M.Sc., Ph.D. (Cantab.), F.N.I. and H. N. Batra, B.Sc., Assoc. I. A. R. I. (1938). Price Re. 1 or 1*s*. 9*d*.

ICAR. 8·20 No. 20. Report on an Enquiry into the Cultivation of Cloves in India. By A. K. Yegna Narayan Iyer, M.A., Dip. Agri. (Cantab.). (1938). Price Rs. 2-4-0 or 3*s*. 9*d*.

ICAR. 8·21 No. 21. Investigations on the Cold Storage of Mangoes. By G. S. Cheema, D.Sc., I.A.S., D. V. Karmarkar, M.Sc., Ph.D., A.I.I.Sc. and B. M. Joshi, M.Sc. (1939). Price Rs. 3-12-0 or 6*s*.

ICAR. 8·22 No. 22. Report on a Village Enquiry regarding Cattle and the Production and Consumption of Milk in Seven Breeding Tracts of India. (1939). Price Rs. 3-8-0 or 5*s*. 6*d*.

ICAR. 8·23 No. 23. The Cold Storage of Fruits and Vegetables. By G. S. Cheema, D.Sc., I.A.S. and D. V. Karmarkar, M.Sc., Ph.D., A.I.I.Sc. (1939). Price As. 10 or 1*s*.

ICAR. 8·24 No. 24. A Brief Survey of some of the Important Breeds of Cattle in India, Part II. (1939). Price Re. 1-6-0 or 2*s*.

ICAR. 8·25 No. 25. The Nutritive Values of Indian Cattle Foods and the Feeding of Animals. By K. C. Sen, D.Sc. (1939). Price As. 7 or 8*d*.

ICAR. 8·26 No. 26. The Progress of Agricultural Science in India during the Past Twenty-five Years. By W. Burns, C.I.E., D.Sc. (Edin.), I.A.S. (1939). Price Re. 1-8-0 or 2*s*. 3*d*.

ICAR. 8·27 No. 27. Definition of Characteristics of Seven Breeds of Cattle of All-India Importance. (1939). Price Re. 1-10-0 or 2*s*. 6*d*.

ICAR. 8·28 No. 28. Bibliography of Soil Erosion, Part I. Indian Section, by R. M. Gorrie, D.Sc. (1939). Price Re. 1-12-0 or 2*s*. 6*d*.

ICAR. 8·29. No. 29. Report on the Prospects of Cinchona Cultivation in India, by A. Wilson, M.A., B.Sc., I. A. S. and T. J. Mirchandani, M.Sc., Ph. D. (*In Press.*)

ICAR. 8·30. No. 30. Biological Notes on Indian Chalcidoidea, by H. S. Pruthi, M.Sc., Ph.D. (Cantab.), F.N.I. and M. S. Mani, M.A. (*In Press.*)

ICAR. 8·31. No. 31. Studies in the Preservation of Fruit and Vegetables : An Investigation on the Methods of Preservation and Standardization of Tomato Ketchup, by Lal Singh, B.Sc. (Hons.), M.Sc. (Calif.) and Girdhari Lal, Ph.D. (Lond.), D.I.C. (*In Press.*)

ICAR. 8·32. No. 32. Studies in the Preservation of Fruit Juices, I. Some Observations on the Preparation and Preservation of Citrus Fruit Squashes, by Lal Singh, B.Sc. (Hons.), M.Sc. (Calif.) and Girdhari Lal, Ph.D (Lond.), D.I.C. (*In Press.*)

6. Annual Report of the Imperial Council of Agricultural Research

ICAR. 12·31 Annual Report of the Imperial Council of Agricultural Research for the years 1929-30 and 1930-31. Price As. 12 or 1*s*. 3*d*.

ICAR. 12·32 Annual Report of the Imperial Council of Agricultural Research for the year 1931-32. Price As. 6 or 8*d*.

ICAR. 12·33 Annual Report of the Imperial Council of Agricultural Research for the year 1932-33. Price As. 6 or 8*d*. (Out of stock.)

ICAR. 12·34 Annual Report of the Imperial Council of Agricultural Research for the year 1933-34. Price Re. 1-8-0 or 2*s*. 6*d*.

ICAR. 12·35 Annual Report of the Imperial Council of Agricultural Research for the year 1934-35. Price Re. 1 or 1*s*. 9*d*.

ICAR. 12·36 Annual Report of the Imperial Council of Agricultural Research for the year 1935-36. Price As. 14 or 1*s*. 6*d*.

ICAR. 12·37 Annual Report of the Imperial Council of Agricultural Research for the year 1936-37. Price Re. 1-2-0 or 2*s*.

ICAR. 12·38 Annual Report of the Imperial Council of Agricultural Research for the year 1937-38. Price As. 12 or 1*s*.

7. Agriculture and Animal Husbandry in India (called Review of Agricultural Operations in India up to 1933)

ICAR. 9·29 Review of Agricultural Operations in India, 1928-29. Price Rs. 3-2 0 or 5*s*. 6*d*. (Out of stock.)

ICAR. 9·31 Review of Agricultural Operations in India, 1929-31. Price Rs. 5 or 8*s*. 3*d*.

ICAR. 9·33 Review of Agricultural Operations in India, 1931-33. Price Rs. 5-12-0 or 9*s* 6*d*.

Agriculture and Animal Husbandry in India, 1933-34 and 1934-35—

ICAR. 13·1·35 Part I.—Crop Production. Price Rs. 4-14-0 or 8*s*.

ICAR. 13·2·35 Part II.—Animal Husbandry. Price Re. 1 or 1*s*. 9*d*.

ICAR. 13·36 Agriculture and Animal Husbandry in India, 1935-36. Price Rs. 4-10-0 or 7*s*. 9*d*.

8. Proceedings of the Board of Agriculture and Animal Husbandry

ICAR. 2 Proceedings of the First Meeting of the Animal Husbandry Wing of the Board of Agriculture and Animal Husbandry held at New Delhi from the 20th to the 23rd February 1933, with Appendices. Price Rs. 5-14-0 or 9*s*. 6*d*.

ICAR. 2·2 Proceedings of the Second Meeting of the Animal Husbandry Wing of the Board of Agriculture and Animal Husbandry held at Madras from the 14th to the 16th December 1936, with Appendices. Price Rs. 3-10-0 or 6*s*. 3*d*.

ICAR. 3 Proceedings of the First Meeting of the Crops and Soils Wing of the Board of Agriculture and Animal Husbandry in India held at Delhi from the 25th February to the 2nd March 1935, with Appendices. Price Rs. 6 or 9*s*. 9*d*.

9. Catalogue of Indian Insects

The following parts have been issued :—

ICAR. 11·1 Acrydidæ (Tettigidæ). By T. Bainbrigge Fletcher. 1921. Price As. 11.

ICAR. 11·2 Culicidæ. By R. Senior-White. 1923. Price Re. 1-10.

ICAR. 11·3 Bombyliidæ. By R. Senior-White. 1923. Price As. 8.

ICAR. 11·4 Trypetidæ (Trypaneidæ). By R. Senior-White. 1924. Price As. 8.

ICAR. 11·5 Nitidulidæ. By S. N. Chatterjee. 1924. Price As. 10.

ICAR. 11·6 Staphylinidæ. By M. Cameron. 1925. Price Re. 1-14.

ICAR. 11·7 Lasiocampidæ. By T. Bainbrigge Fletcher. 1925. Price As. 12.

ICAR. 11·8 Amatidæ (Syntomidæ). By T. Bainbrigge Fletcher. 1925. Price As. 12.

ICAR. 11·9 Zygænidæ. By T. Bainbrigge Fletcher. 1925. Price Re. 1-5.

ICAR. 11·10 Stephanidæ. By G. R. Dutt. 1925. Price Re. 1-2.

ICAR. 11·11 Brenthidæ. By Richard Kleine. 1926. Price Re. 1-2.

ICAR. 11·12 Tabanidæ. By R. Senior-White. 1927. Price Re. 1-12.

ICAR. 11·13 Cicindelidæ. By Mercia Heynes-Wood and Cedric Dover. 1928. Price Rs. 2-8.

ICAR. 11-14 Palpicornia. By A. d'Orchymont. 1928. Price Rs. 2-8.

ICAR. 11-15 Cecidomyidæ. By R. Senior-White. 1928. Price As. 7.

ICAR. 11-16 Cosmopterygidæ. By T. Bainbrigge Fletcher. 1928. Price As. 10.

ICAR. 11·17 Yponomeutidæ. By T. Bainbrigge Fletcher. 1928. Price As. 8.

ICAR. 11·18 Carabidæ. By H. E. Andrewes. 1931. Price Rs. 8-10.

ICAR. 11·19 Gyrinoidea. By Georg Ochs. 1930. Price As. 12.

ICAR. 11·20 Alucitidæ (Pterophoridæ). By T. Bainbrigge Fletcher. 1931. Price Rs. 1-6.

ICAR. 11·21 Lycidæ. By R. Kleine. 1931. Price Re. 1-2.

ICAR. 11·22 Phaloniadæ and Chlidanotidæ. By T. Bainbrigge Fletcher. 1931. Price As. 6.

ICAR. 11·23 Chalcidoidea. By M. S. Mani, M.A. (Res.), 1937. Price Rs. 3-2 or 5*s*. 6*d*.

10. Miscellaneous

VRI. 1 — A Description of the Imperial Institute of Veterinary Research, Muktesar, and its Sub-station, the Imperial Veterinary Serum Institute, Izatnagar. By F. Ware, F.R.C.V.S., I.V.S. Price Re. 1-4-0 or 2*s*.

ARI. 7. 187 — The Production of Cigarette Tobacco by Flue-curing. By F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S. and Kashi Ram. *Imp. Inst. Agri. Res., Pusa, Bull.* No. 187. Reprinted (1935). Price Re. 1 or 1*s*. 9*d*.

ICAR. 16 — A Handbook of Statistics for use in Plant Breeding and Agricultural Problems. By F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S. Price Re. 4-6-0 or 7*s*. 3*d*.

ICAR. 7 — Report on the Work of the Imperial Council of Agricultural Research in Applying Science to Crop Production in India. By Sir John Russell, D.Sc., F.R.S. Price Re. 1-14-0 or 3*s*. 3*d*.

ICAR. 18 — Report on the Development of the Cattle and Dairy Industries of India. By Norman C. Wright, M.A., D.Sc., Ph.D. Price Re. 1-8-0 or 2*s*. 6*d*.

Copies of the above publications can be had from the Manager of Publications, Civil Lines, Delhi and from any of the Agents, a list of whom will be found on the inside page of the front cover. Prices are inclusive of packing and Indian postage.

Purchasers not residing in Asia should apply to the High Commissioner for India, India House, Aldwych, London.

Note.—When indenting please give only the symbol preceding the name of the publication.

GIPD—S3—646 IC of AR—24-4-39—800.

NOTICE

The Indian Journal of Agricultural Science

A Bi-monthly Scientific Journal of Agriculture and the Allied Sciences, mainly devoted to the publication of the results of original research and field experiments

EDITORIAL COMMITTEE

P. M. KHAREGAT, C.I.E., I.C.S., *Vice-Chairman, Imperial Council of Agricultural Research*

W. BURNS, C.I.E., D.Sc., I.A.S., *Agricultural Commissioner with the Government of India*

F. WARE, C.I.E., F.R.C.V.S., F.N.I., I.V.S., *Animal Husbandry Commissioner with the Government of India*

RAO BAHADUR B. VISWANATH, F.I.C., F.C.S., *Director, Imperial Agricultural Research Institute*

J. R. HADDOW, B.Sc., M.R.C.V.S., D.V.S.M., I.V.S., *Director, Imperial Veterinary Research Institute, Mukteswar*

J. C. GHOSH, D.Sc., *Professor of Chemistry, University of Dacca, Dacca*

JAMES N. WARNER, M.Sc., *Offg. Professor of Animal Husbandry and Dairying, Allahabad Agricultural Institute, Allahabad*

S. BASU, I.C.S., *Secretary, Imperial Council of Agricultural Research*

The Editorial Committee, in the work of examining papers submitted for publication, is assisted in an honorary capacity by a large number of scientists working in various parts of India.

1. Editorial communications should be addressed to the Secretary, Imperial Council of Agricultural Research, Publication Section, New Delhi.

2. Contributors are entitled to receive 30 free reprints of their contributions; but additional copies can be had, provided notice is given at the time of returning the proofs. The rates for the supply of every 50 additional reprints, or part thereof, are as follows:—

As. 8 per type page, As. 15-3 per black-and-white plate when printed on one side of the paper only, Re. 1-5 per black-and-white plate when printed on both sides of the paper and Rs. 2-8 per three-coloured plate.

3. All communications regarding subscription and advertisements should be addressed to the Manager of Publications, Civil Lines, Delhi.

CONTENTS

(Vol. IX, Part II)

April, 1939

The Editorial Committee of the Imperial Council of Agricultural Research, India, takes no responsibility for the opinions expressed in this Journal.

		Page
Original articles—		
The Genus *Fusarium*, I. Known Occurrence in India	*G. Watts Padwick*	171
Studies in Indian Chillies: (5) Inheritance of Anther Colour and its relation to Colour in Petal and Node in *Capsicum annuum* L.	*R. B. Deshpande, M.Ag. (Bom.), Assoc. I.A.R.I.*	185
Studies on Quality in Rice—		
III. *In vitro* Digestibilities of different Varieties of Rice	*A. Sreenivasan, M.A., D.Sc., A.I.C. and K. Venkata Giri, M.Sc., Ph.D., A.I.I.Sc.*	193
IV. Storage Changes in Rice after Harvest	*A. Sreenivasan, M.A., D.Sc., A.I.C.*	208
Entomological Investigations on the Leaf-curl Disease of Tobacco in Northern India, III. The Transmission of Leaf-curl by White-fly, *Bemisia gossypiperda*, to Tobacco, Sann-hemp and a New Alternate Host of the Leaf-curl Virus (with Plates VII-X and one text-figure)	*Hem Singh Pruthi, M.Sc., Ph.D. (Cantab.) F.N.I. and C. K. Samuel, B.Sc.*	223
The Phytophagous Chalcidoid, *Eurytoma samsonovi* Vas., a Serious Pest of Apricot and some other Fruits in India (with Plate XI)	*Hem Singh Pruthi, M.Sc., Ph.D. (Cantab.), F.N.I. and H. N. Batra, B.Sc., Assoc. I.A.R.I.*	277
Studies in the Periodic Partial Failures of American Cotton in the Punjab—		
I. Early Breakdown of the Photosynthetic Apparatus (with Plates XII-XIV)	*R. H. Dastur*	285
II. Formation and Accumulation of Tannins in Leaves (with Plates XV-XVII)	*R. H. Dastur*	291
Variations in the Measurable Characters of Cotton Fibre, I. Variation with respect to the Length of the Fibre (with eight text-figures)	*R. L. N. Iyenger, M.Sc.*	305
Studies on the Root-rot Disease of Cotton in the Punjab, VI. Chemical Composition of Healthy and Diseased Cotton Plants	*R. S. Vasudeva, B.Sc., Ph.D. (Lond.), D.I.C. (Lond.) and Mohammad Rafique, M.Sc.*	331
Note		343
Reviews—		
Soilless Growth of Plants		353
Plant Injection for Diagnostic and Curative Purposes		354